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Virus Receptors: Implications for Pathogenesis and the Design of Antiviral Agents

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INTRODUCTION

A virus initiates infection by attaching to its specific receptor on the surface of a susceptible host cell. This prepares the way for the virus to enter the cell. In at least some cases, the receptor also plays an important role in entry per se in addition to its role in virus binding. Consequently, the expression of the receptor on specific cells or tissues in the whole host is a major determinant of the route of virus entry into the host, the pattern of virus spread in the host, and the resulting pathogenesis. In addition to the fundamental biological and clinical importance of virus receptors, they are also of potential practical significance because the rational design of drugs that inhibit virus-receptor interactions at the points of virus attachment or entry provides a novel approach to the therapeutic treatment of virus diseases.

Despite the importance of virus receptors, the identities and host cell functions of relatively few are presently known or widely accepted. Also, in most cases, there is only scant information available concerning the molecular mechanisms that underlie virus binding and entry. In addition, these mechanisms may be complex, involving multiple sites or factors on both the virus and the cell. Indeed, the earlier view that virus-receptor interactions resemble the interactions of simple ligands with their receptors, defined largely by factors such as ionic strength, pH, and temperature (for an example, see reference 137), has gradually given way to a view of a more dynamic multistep process (89). For some viruses, this means that initial binding might be followed by a secondary binding step involving other sites or components on the virus and the cell. The secondary interactions might strengthen adhesion and enable penetration either by fusion or endocytosis. Each of these steps might entail conformational changes in viral and cellular components that are necessary to promote subsequent stages of binding and entry. These complexities largely account for why much remains to be done before the details and sequences of events in virus binding and entry are completely understood.

This review emphasizes human immunodeficiency virus (HIV), the rhinoviruses, and several of the herpesviruses. The coronaviruses are also considered briefly. These viruses are given special consideration here because of their medical significance. Furthermore, because of their medical significance, they have been studied extensively, and relatively more information concerning their receptors is available. This knowledge offers insights into patterns of pathogenesis, and in the cases of HIV and the rhinoviruses, ingenious approaches to receptor-based therapeutic strategies are under development and in clinical trials. HIV and the rhinoviruses are also considered in the broader contexts of their larger families, the retroviruses and picornaviruses, respectively. This is for the purposes of comparison and to illustrate general principles of virus-receptor interactions.

Since this review is limited to the aforementioned viruses, attention is directed to several recent reviews that consider other viruses and virus families, including African swine fever virus (11), the paramyxoviruses (125, 126), the reoviruses (195), hepatitis B virus (131), and influenza virus (93). Several other reviews and conference reports complement and expand on the material presented here (48, 89, 137, 234). A review by Marsh and Helenius (144) considers in greater detail the mechanisms by which viruses enter cells. A recent review by Dimmock (59) discusses neutralization of viruses.

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RETROVIRUS RECEPTORS

The HIV Receptor Is CD4

Retroviruses are associated with a wide range of diseases in vertebrates, including cancer, neurological disorders, and AIDS. Two distinct groups of retroviruses which infect humans are recognized. One group includes the leukemia viruses, human T-cell leukemia virus type I (HTLV-I) and HTLV-II. The other group includes the immunodeficiency viruses, HIV type 1 (HIV-1) and HIV-2. The immunodeficiency viruses belong to the lentivirus family of slow viruses, which includes visna virus of sheep and the equine infectious anemia virus.

Genetic and interference studies (see below) have shown that different strains of retroviruses use different host cell surface molecules for their receptors. Considering that HIV is one of the most recently discovered viruses, it is noteworthy that the interaction of HIV with its receptor is perhaps the best characterized of all virus-receptor interactions. Because of the importance of the AIDS epidemic, the relatively extensive knowledge of the interaction of HIV with its receptor, and the development of AIDS therapies based on that interaction, HIV is discussed at length in this review.

The identification of the CD4 glycoprotein as the HIV receptor was made independently by two groups (51, 120). CD4 is present on the surfaces of those T lymphocytes that have helper or inducer functions. Those lymphocytes interact with target cells that present antigen in association with major histocompatibility complex (MHC) class II proteins. CD4 is believed to increase the affinity of the T-cell receptor for MHC class II proteins on the antigen-presenting cell, perhaps forming part of the receptor for the complex of antigenic peptide and MHC class II proteins (106). Furthermore, CD4 may contribute directly to the signal transmission which results in T-cell activation (94). The activated $CD4^+$ cells secrete lymphokines that help to activate other cells, in particular, B cells and macrophages. CD4 is also present on cells of the monocyte/macrophage lineage.

Klatzmann et al. (120) considered the possibility that CD4 might be the HIV receptor because $CD4^+$ (T4) lymphocytes are depleted in AIDS patients and because HIV appeared to have a specific tropism for those cells. The major finding of that group was that HIV infection of $CD4^+$ lymphocytes could be blocked by preincubating the cells with monoclonal antibodies (MAbs) against CD4. These MAbs presumably blocked infection by binding specifically to the virus receptor, thereby blocking binding by the virus. The blocking effect of the anti-CD4 MAbs is indeed specific since MAbs against other cell surface proteins did not block infection. The finding described above shows how knowledge of pathology and virus tropism provided the initial clue to the identity of the HIV receptor. This, in turn, led to further understanding of the virus tropism and pathogenesis.

Dalgliesh et al. (51) followed a different approach. Since there was no quantitative infectivity assay for HIV, they tested the susceptibility of cells to infection with pseudotypes of vesicular stomatitis virus (VSV). These particles contain the VSV genome within an envelope that includes HIV envelope glycoproteins. They are produced when cells infected with HIV are superinfected with VSV. Pseudotype virions, which are selected for resistance to neutralization by anti-VSV antiserum, do not contain VSV envelope proteins. However, they may have sufficient HIV envelope glycoprotein to infect cells that express the HIV receptor. Indeed, their host range should be restricted to cells expressing the HIV receptor. The VSV genome then replicates in those cells to produce nonpseudotype VSV virions, which are readily detected. Receptors for the pseudotype virions were detected only on cells expressing CD4. Next, a blind study was conducted in which more than 150 MAbs against T-cell surface antigens were screened for their ability to inhibit the cell fusion (syncytia) induced when uninfected cells bearing HIV receptors are mixed with cells producing HIV. (Note that syncytium formation is believed to mimic viral entry [see below] and is thought to be an important mode of viral spreading in vivo.) The anti-CD4 MAbs specifically blocked syncytium formation. Furthermore, the anti-CD4 MAbs specifically blocked infection of the $CD4^+$ cells by the VSV pseudotype virions.

Both of the groups mentioned above (51, 120) realized that the evidence then available did not provide compelling proof that cell surface expression of CD4 is sufficient to facilitate viral attachment and entry. One group stated that the "findings strongly suggest that the T4 glycoprotein is at least associated with all or part of the receptor" (120). The other group cautiously titled their paper "The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus" (51). The finding that transfection of a functional CD4 gene into $CD4^-$, HIV-resistant human epithelial cells restores susceptibility to infection (140) may have seemed to provide the compelling proof. However, as described below, whereas cell surface CD4 may be sufficient for HIV to adsorb to cells, it is not sufficient to ensure HIV entry into the cells.

The following shows that cell-specific factors in addition to CD4 are necessary for HIV to enter cells. First, note that after HIV binds to CD4 at the cell surface, the HIV envelope fuses with the cell membrane, thereby releasing the virion core into the cytoplasm (see below). Several $CD4^-$, HIV-resistant human cell lines could be infected by HIV after they were engineered to express human CD4. In contrast, cell lines of murine, feline, rabbit, and simian origins could not be infected with HIV, even though they were also engineered to express human CD4 at their surfaces. In all cases, the block to infection appeared to be at the level of virus entry. As noted above, HIV envelope-mediated fusion can be monitored by observing the related process in which cells expressing HIV envelope glycoproteins specifically fuse to $CD4^+$ cells, resulting in the formation of multinucleate syncytia. Although murine cell lines which have been engineered to express human CD4 are resistant to the fusogenic effect of HIV, heterokaryons made from $CD4^+$ murine cells and $CD4^-$ human cells do undergo HIV-mediated fusion (60). Thus, the fusion-resistant phenotype of $CD4^+$ murine cells can be complemented by human cell factors. This implies that human cell surface factors in addition to CD4 are necessary for entry of HIV.

These additional human cell surface factors are probably not present on all human cells, since not all human cell lines can be infected with HIV after transfection with a CD4 expression vector (39). HIV is able to bind to the CD4 expressed by these nonsusceptible transfected human cells but is probably unable to fuse with their plasma membranes as implied by assays for syncytium formation.

Characterization of the cell surface factors that are required in addition to CD4 for HIV infectivity has potential importance for the development of new drugs designed to impede the spread of HIV in the body (see below). It would also lead to an understanding of why HIV is able to infect only some $CD4^+$ cells in vivo. It was recently claimed that a cell surface protein known as CD26 is the coreceptor for HIV (33). This was based largely on the finding that coexpression of human CD4 and CD26 in mouse NIH 3T3 cells rendered them permissive to HIV. Of further interest, CD26 is a protease that recognizes a specific motif contained in the V3 loop of the viral envelope glycoprotein gp120. The V3 loop of gp120 is critical

for HIV infection (see below). Entry of HIV into lymphoid cells was blocked by antibodies specific for CD26 and by specific inhibitors of the protease activity (33). A preliminary discussion of this work is contained in reference 16.

The CD4 protein is a single 55-kDa cell surface polypeptide. Its extracellular region contains 372 amino acids that make up four tandem immunoglobulin (Ig)-like domains (Fig. 1). Ig-like domains are characterized by two anti-parallel β-pleated structures (one containing four chains and the other containing three chains) held together by an intrachain disulfide bond. Of particular interest are the loops between the chains and between the sheets. At the variable regions of antibody molecules, three of these loops come together to form antigen-binding sites (see below). The most amino-terminal Ig-like domain (domain 1) of CD4 is indeed homologous to the variable regions of antibody molecules. The three other Ig-like domains show different degrees of similarity to antibody-variable (domain 3) or to truncated constant (domains 2 and 4) regions (reviewed in reference 12). Amino acid substitutions in either domain 1 or domain 2 affect the interaction of CD4 with MHC class II molecules (42).

Molecules that contain Ig-like domains constitute a family of proteins that have many different functions. However, like CD4, they generally have a recognition or binding role at the cell surface. It is noteworthy that several other viruses, all unrelated to HIV, also use proteins of the Ig superfamily for their receptors. The significance of this is discussed later.

Studies with truncated derivatives of CD4 (12) and human/mouse chimeric CD4 proteins (127) show that CD4 domain 1 is critical for recognition by HIV. In further analysis of truncated derivatives and single amino acid substitutions (12), the primary HIV-binding site was localized to a region encompassing amino acids 40 to 48 in domain 1. This region overlaps a protruding loop region that is structurally homologous to the second complementarity-determining region (CDR2) of Ig light chains. (The specificity of antigen recognition by Ig is determined largely by the structure of the three complementarity-determining regions [CDR1, CDR2, and CDR3] within the variable domains. These regions, which are in the separate loops, come together to form a surface complementary to that of antigen.)

The recognition by HIV of a small loop on CD4 may have important implications concerning the ability of HIV to evade neutralizing antibodies. As discussed below regarding the receptor for rhinoviruses, it was suggested that, whereas the canyonlike receptor-binding sites on rhinoviruses are large enough to permit penetration by an exposed loop of the Ig-like domain of their receptor, they are too small to be accessible to the entire antigen recognition regions of antibody molecules (47, 186). Thus, the receptor-binding sites on the virus would be nonimmunogenic. This would enable the virus to undergo antigenic variation to escape neutralization while being able to conserve its receptor specificity. If the region of gp120 (the HIV envelope glycoprotein that interacts with CD4; see below) is a groove rather than a flat surface, then the groove must be at least 25Å (2.5 nm) long and 12Å (1.2 nm) wide, as implied by the structure of CD4 (228).

HIV interacts with its receptor via its envelope glycoproteins. These glycoproteins, gp120 and gp41, are synthesized initially as a precursor (gp160), which is cleaved by cellular enzymes to a noncovalently associated gp120-gp41 complex. The transmembrane gp41 anchors the complex to the viral envelope, whereas the external gp120 contains the CD4-binding activity.

As noted above, after binding to the target cell surface, HIV enters the cell by direct fusion between the virion envelope and the plasma membrane. The mechanism of the fusion for HIV or any other retrovirus is not known in detail. CD4 probably plays a role in this process after serving as the surface attachment protein. This is suggested by the following. Anti-CD4 MAbs, which bind to CD4 domain 1, may block infection by competitively inhibiting virus attachment. In contrast, an MAb, 5A8, which is reactive with CD4 domain 2, inhibits HIV infection while not blocking HIV binding (160). MAb 5A8 is believed to block infection by interfering with the conformational changes in the viral envelope glycoproteins and/or CD4, which are induced by binding and which may be necessary for fusion. Consistent with this, binding of soluble CD4 (sCD4) to virions leads to the enhanced exposure of specific regions on gp120 and gp41 (43, 194), as shown by MAb binding and sensitivity to proteases. Furthermore, binding to CD4 leads to the dissociation of gp120 from some strains of HIV (160). These effects of sCD4 are blocked by MAb 5A8 (160).

The results described above show that separate regions of CD4 are likely responsible for HIV binding and membrane fusion. As noted, the entire binding site for HIV may be included within residues 40 to 48 in the CDR2-like loop (12, 191, 228). In contrast, residues affecting syncytium formation were found to be clustered about residue 87 in the spatially separated CDR3-like loop (34, 181, 191, 228). Consequently, although the region about residue 87 may not be important for the initial binding of HIV, the CDR3-like loop may be necessary for subsequent interactions with gp120 which are necessary for fusion. Note that more recent findings challenge the notion that the CD3 region is involved in membrane fusion (see references 28 and 158 and references therein).

Two lines of evidence imply that HIV enters cells by direct membrane fusion rather than by receptor-mediated endocytosis. First, as noted above, the membranes of HIV-infected cells fuse with the membranes of uninfected $CD4^+$ cells. Second, the low pH of endosomes is not required for infection, as might be the case if HIV entered cells by receptor-mediated endocytosis. Nevertheless, CD4 appears to play a role in HIV entry distinct from merely serving as the attachment protein for the virus. One possibility is that HIV entry might somehow depend on the internalization of CD4. In this regard, note that CD4 undergoes endocytosis following T-cell activation (2, 99). Also, CD4 internalization is dependent on the activation of protein kinase C and subsequent phosphorylation of CD4 (2, 99, 141). Thus, it is interesting that HIV binding was also reported to induce a CD4-mediated signal resulting in phosphorylation of CD4 via a protein kinase C-dependent pathway (63, 99). However, other studies did not find evidence for an HIV-induced CD4-mediated T-lymphocyte signal transduction pathway (86, 98, 100), HIV entry was not found to be inhibited by agents that block signal transduction (173), and HIV was not found to induce the internalization of CD4 (173). Most critically, cells expressing mutations in the cytoplasmic domain of CD4, which severely diminish the ability of CD4 to undergo endocytosis, are readily infected by HIV (141). These last findings are consistent with the infectious entry pathway for HIV proceeding via membrane fusion rather than by receptor-mediated endocytosis that might require continued association between the virus and CD4 during entry.

The results described above, when considered together, show that multiple domains of CD4 as well as at least one other cell surface factor interact with HIV in a complex way that is necessary for the virus to bind and enter into target cells. Also implicit in the results described above is the idea that the envelope glycoproteins of the virus may have a role in membrane fusion distinct from their role in high-affinity binding. Indeed, mutants of gp120 which have severely impaired fuso-

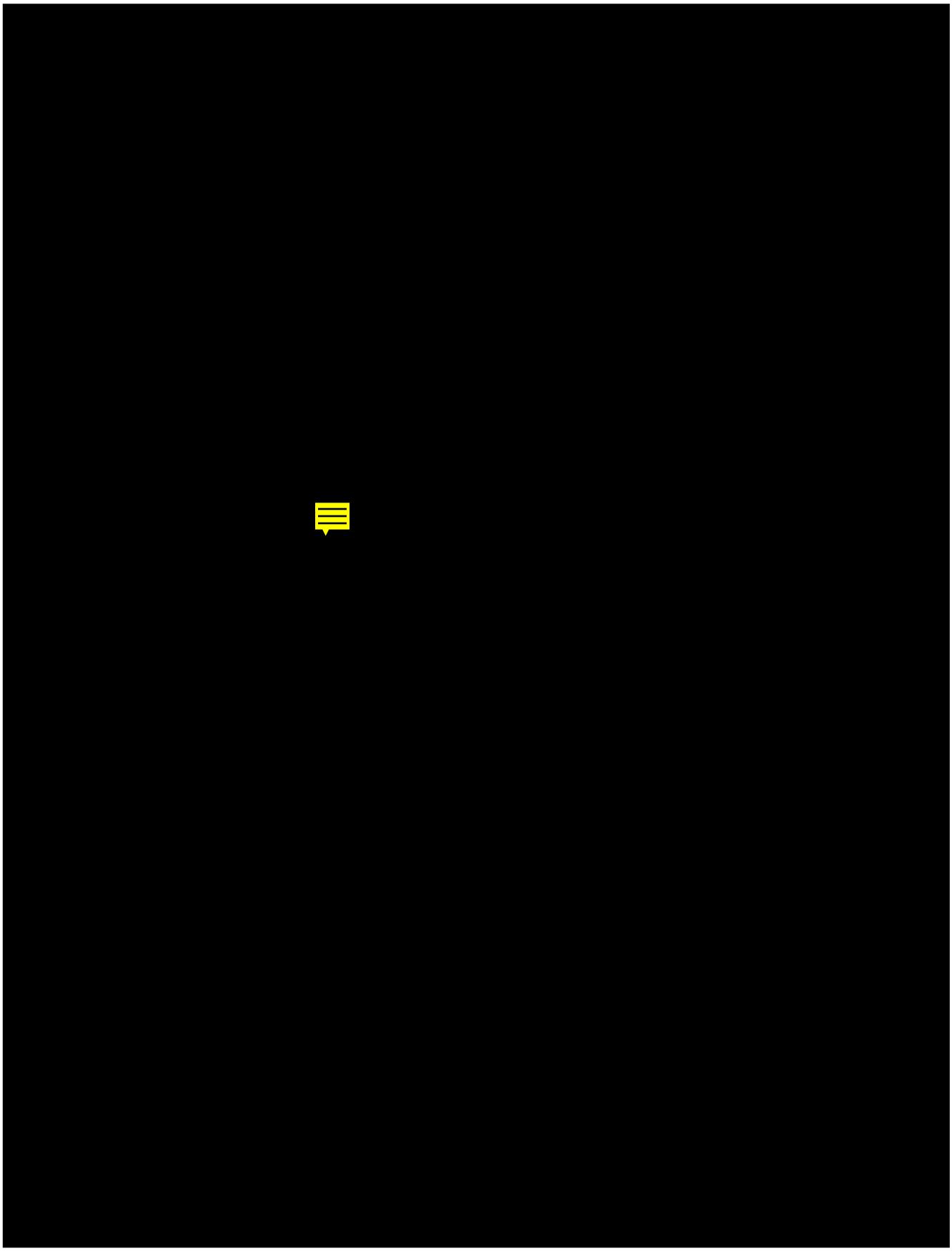

FIG. 1. (a) Backbone representation of CD4 (residues 1 to 182). Domain 1 is in red, domain 2 is in blue; β strands are indicated by letters, separately in each domain. Strand A of domain 2 is continuous with strand G of domain 1. Note that domains 1 and 2 are related by a rotation of approximately 160° and a translation along the axis of the molecule. Disulfide bonds are shown as solid lines; only the trace is visible of the disulfide bond between strands B and F in domain 1. (b) Solid representation of CD4 (residues 1 to 182) in an orientation similar to that shown in panel a. The C″ ridge of domain 1, implicated in the binding of HIV gp120, is highlighted. (c) Representations of domains 1 and 2 oriented to show the similarity of their folded structures. First and last residues in each strand are indicated by single-letter code and sequence numbers. Reprinted with permission from reference 228.

genic activity and infectivity, while binding normally to CD4, have been generated by site-directed mutagenesis (80, 104, 174, 224, 237). Furthermore, the amino terminus of gp41 has also been shown to be important for promoting fusion (see below).

The CD4-binding domain of gp120 is encompassed by residues 404 to 447 (129). In contrast, point mutations in gp120, which impair infectivity and cell-cell fusion without affecting binding to CD4, have been localized to residues 296 to 331 (104, 207). These residues are thought to form a loop structure (the V3 loop) by disulfide linkage of two cysteine residues. The region of the V3 loop that is essential for fusion and infectivity has been further localized to a strongly conserved tetrapeptide (residues 312 to 315) located at the center of the loop (68, 104).

The V3 loop may act in membrane fusion by interacting with a particular region of CD4. In support of this, synthetic peptides containing V3 sequences bind to cell surface CD4 (14). Conversely, a synthetic peptide based on a sequence of CD4 domain 1 may bind to V3 (19).

Further evidence that the V3 loop of gp120 interacts with CD4 comes from studies of the neutralization of HIV with sCD4. (The therapeutic use of sCD4 is discussed in detail below.) A comparison of sCD4-sensitive and sCD4-resistant HIV strains, and their recombinants, showed that the region of gp120 important for determining sensitivity to sCD4 neutralization includes the V3 loop but does not include regions of gp120 that are most important for CD4 binding (170). It was suggested that the interaction of CD4 with the CD4-binding region of gp120 might cause a conformational change in gp120 that permits the V3 loop to interact with a second site on CD4. It is also possible that V3 interacts with other cell surface factors instead of, or in addition to, CD4.

The V3 loop is of further interest because it is hypervariable and also contains the principal determinants for type-specific neutralization by anti-HIV antibodies (68, 128, 190). Furthermore, fusion is also blocked by antibodies specific for V3 loop determinants, even though those antibodies do not block the binding of gp120 to CD4 (207).

Yet another region of gp120 may be important in HIV entry. Antisera against synthetic peptides corresponding to residues 244 to 264 (95), or a point mutation at residue 259 (179), did not block binding to CD4 but markedly affected the infectivity of T cells by some strains of HIV. Deletions or insertion mutations in this region can also affect HIV binding to CD4 as well as fusion (123).

Although not unexpected, it is worth noting that the regions of gp120 that are important in its interaction with CD4 tend to be highly conserved among HIV isolates.

The other HIV envelope glycoprotein, gp41, is most likely the actual fusion glycoprotein. Several lines of evidence support this. First, mutations in sequences that encode the hydrophobic amino terminus of gp41 interfere with fusion while not affecting binding (61, 123). Second, there is the analogy with other enveloped viruses, such as the paramyxoviruses and influenza, in which separate envelope glycoproteins are involved in the binding and fusion reactions. In these viruses, the fusion protein is also an integral envelope protein generated by the proteolytic cleavage of a precursor polyprotein. Furthermore, as in the case of gp41, it is the hydrophobic amino termini of those fusion proteins that impart their fusogenic potential. A hydrophobic sequence at the amino terminus of gp41 is highly conserved among various HIV isolates (76). This sequence resembles similarly located sequences in the fusion proteins of paramyxoviruses.

Taken together, the findings described above show that HIV binding and entry are dependent on the interactions of several domains on gp120 with several domains on CD4 as well as on the activity of gp41. Presumably, when the gp120/gp41 complex binds to CD4 via gp120, conformational changes occur in CD4 and in the viral envelope glycoproteins. These changes expose the amino terminus of gp41, which can promote fusion. Furthermore, at least one other cellular protein (possibly CD26) plays an unknown, but necessary, accessory role in virus entry.

HIV infects monocytes, macrophages, and other cells (see below) in addition to $CD4^+$ lymphocytes. Studies examining the role of CD4 in HIV infection of macrophages have yielded conflicting results. Although monocytes/macrophages can express CD4 (45), some reports show that monocyte-derived cells can be infected with HIV while not expressing CD4 at the time of infection (41, 217). In contrast, other studies show that infection of monocytes can be blocked with sCD4 and with anti-CD4 MAbs (41, 45). The normal function of CD4 on monocyte-derived cells is not known.

A region of gp120 that encompasses the V3 loop determines HIV tropism for macrophages. This was shown by studies with recombinants of HIV strains that differ in their abilities to infect these cells (171, 201). This region of gp120 spans residues 202 to 358. As noted above, this region was implicated in fusion and possibly binding as well. Many HIV strains grow slowly in monocytes/macrophages that express high levels of CD4. Recent studies show that viral entry, which is inefficient in these cells, is the major limiting event (171). Regardless of these findings, the mechanism by which a particular gp120 sequence, especially one containing the V3 loop, preferentially enhances infection of monocytes and microglial cells is unclear.

The correspondence between a genetic property of HIV and the tropism of the virus for cells of the mononuclear phagocyte system, which has as its basis the virus-receptor interaction, is relevant to the neurologic and pulmonary sequelae of AIDS. Cells of the mononuclear phagocyte system are the major cells that produce HIV in many tissues, including the central nervous system (CNS) (77) and lungs (124). Furthermore, AIDS dementia is correlated with infection of macrophages and/or microglia in the brain. Thus, the emergence of HIV variants that express enhanced tropism for macrophages can profoundly affect the disease course in AIDS.

Alternative Receptors for HIV

It is now widely accepted that CD4 is the main receptor for HIV, determining viral tropism for $CD4^+$ lymphocytes and, possibly, monocytes and macrophages as well. However, HIV is also known to infect many $CD4^-$ human cell lines, including

those derived from colorectal carcinoma (3), rhabdomyosarcoma (41), bone marrow precursor cells (65), chondrocytes (103), synovial cells (103), hepatoma (35), glioma (38, 40, 41, 56, 85), and neuroblastoma (134). Evidence that infection of these cells is independent of CD4 is based on the absence of detectable levels of CD4 at the cell surface or of intracellular CD4 mRNA. Also, infection cannot be blocked by sCD4 or by anti-CD4 MAbs. However, as might be expected, infection of these other cells by HIV is much less efficient than infection of $CD4^+$ lymphoid cells. Thus, whereas the cellular tropism of HIV is not exclusively dependent on CD4, the role of CD4 as the primary receptor for HIV is not in dispute. HIV has been shown to infect certain nonlymphoid cells or tissues in vivo, including the colon, rectum, duodenum, cervix, retina, brain, and megakaryocytes (163, 179, 180, 236, 246).

The above findings may be extremely important from a clinical point of view. The colorectal mucosal epithelium is a major route of HIV infection. Furthermore, HIV infection of epithelial intestinal cells induces a defect of brush border assembly (62), which might account for the malabsorption observed in some AIDS patients (122). The finding that HIV can be isolated from the synovial fluid of patients with severe joint pain (239) suggests that infection of synovial cells or chondrocytes by HIV may contribute to the rheumatological symptoms in some AIDS patients.

The role of CD4 in CNS infection is not clear. This is because the most commonly infected cells in the brains of patients with HIV-induced neurologic syndromes are macrophages (75, 121, 213, 236), which probably express CD4 on their surfaces. Nevertheless, scattered astrocytes, neurons, and brain endothelial cells also may be infected (75, 182, 236). These cells are not likely to express CD4, as indicated by studies of HIV-susceptible human neural cells in culture (41, 85).

The findings described above underscore the need to define the full spectrum of target cells in HIV infection and to identify the cell surface determinants of the HIV tropism for those cells. The latter may be important in the development of therapeutic agents which are based on virus-receptor interactions and to predict possible limitations to therapies based on the interaction between gp120 and CD4 (see below).

Some progress has been made in the identification of alternate receptors for HIV. The screening of MAbs against neural cell surface components for their abilities to block HIV infection of neural cell lines led to the identification of the sphingolipid galactosyl ceramide (GalC) as a likely receptor for HIV on neural cells (84). Specifically, anti-GalC antibodies inhibited HIV infection of neural cells. Also, gp120 could specifically bind to GalC. Anti-GalC, but not anti-CD4, antibodies also inhibited infection of human colon epithelial cells (242). Note that GalC is not detected on lymphoid cells, and anti-GalC antibodies did not inhibit infection of those cells (242). These findings suggest the likelihood that HIV infections of cells of the nervous system and gastrointestinal tract have a common pathway of virus entry involving GalC, or a closely related molecule, rather than CD4.

A particularly troubling finding is that HIV can efficiently infect cells (macrophages, lymphocytes, and human fibroblastoid cells) expressing Fc receptor in the presence of anti-HIV antibodies through a mechanism independent of CD4 (97). Antibodies that actually enhance HIV infectivity in vitro have been found in the blood of infected individuals and in infected or immunized animals (97, 182a, 214). These enhancing antibodies have been invoked to explain at least in part the apparent lack of protection of neutralizing HIV antibodies in vitro (182a). Moreover, HIV strains that are not tropic for macrophages can productively infect macrophages in the presence of enhancing antibodies (97). The findings described above raise serious concerns about the likelihood of developing safe and effective vaccines which do not actually promote the spread of HIV to, or within, some individuals. Moreover, the existence of an alternative pathway to HIV infection, independent of CD4, suggests that therapeutic strategies based on blocking the HIV-CD4 interaction may not be sufficient (see below).

Note that some other viruses, in addition to HIV, may also replicate in macrophages. Because macrophages have Fc receptors, viruses coated with antibody may be more readily taken up by these cells. If macrophages happen to be permissive for these viruses, the result might be the amplification of viremia rather than clearance of virus. Dengue fever virus provides another example in which antibody may play an important role in actually disseminating the infection (83).

CD4-Based AIDS Therapies

An important impetus to the analysis of virus receptors is the hope that the knowledge gained might lead to the development of clinically effective antiviral agents. In the case of HIV, one approach has been to create soluble forms of CD4 that might block HIV infection of cells. It was presumed that sCD4 would act by competing with the binding of the virus to CD4 at the cell surface. Recombinant truncated forms of CD4 had to be developed for this purpose since the complete molecule with its transmembrane domain is insoluble in serum.

As noted above, recombinant sCD4 (rsCD4) indeed efficiently blocks infection of human cells by laboratory strains of HIV in vitro and is now being evaluated for its potential in AIDS therapy. Unfortunately, the phase I and phase II clinical trials did not provide any consistent evidence that rsCD4 might have an antiviral effect in vivo (as assessed by serum levels of HIV antigen and HIV virus titers in the blood). In one early study, there was an indication that daily doses of rsCD4 (30 mg) might cause a decline in HIV antigen levels in patients (197). However, these results were not confirmed in subsequent studies (49, 113, 171).

rsCD4 did not have an antiviral effect in vivo despite the fact that the concentrations of rsCD4 in serum were in the range required to inhibit HIV replication in vitro (i.e., 5 to 80 ng/ml) (110). However, important differences between in vitro and in vivo conditions indicate that much higher levels of serum rsCD4 may be required for clinical efficacy. One important factor is that primary HIV isolates from infected persons are less sensitive to inhibition by rsCD4 than laboratory-adapted strains (49, 154). The promising in vitro studies that preceded the clinical trials were done with HIV isolates that had been propagated in cell culture for many years.

Reexamination of rsCD4 sensitivity in vitro, by comparing primary patient isolates with laboratory strains, showed that 200 to 2,700 times more rsCD4 was required to neutralize primary isolates than to neutralize laboratory-adapted strains (49). In this regard, the genetic instability of HIV and the heterogeneity among HIV isolates are well known. The virus is present in infected persons as a population of genetically diverse variants termed a quasispecies (153). Consequently, the HIV population in infected individuals cannot be represented by any one isolate. Furthermore, in vitro cultivations of primary isolates may select for variants that are only a minor component of the quasispecies in vivo.

The basis for the differences in sCD4 sensitivity of different HIV isolates is not entirely clear. However, the following is known. The resistant isolates do not use an alternative mechanism to gain entry into cells since the infectivity of all isolates

is blocked by anti-CD4 MAbs (49). It is probably important that sCD4 binds with 10- to 30-fold-higher affinity to virions of laboratory strains than to virions of primary isolates (160). This was somewhat surprising since sCD4 binds equally well to soluble gp120 from either laboratory strains or clinical isolates (13, 160). The explanation for this discrepancy might involve the association of gp120 with gp41 on virions. This association on primary HIV isolates may be such as to somewhat impair the interaction of gp120 with CD4 (160).

Another potentially important difference between laboratory strains and primary isolates is that the former are much more sensitive to sCD4-induced shedding of gp120 (160). It was suggested that low concentrations of sCD4 block infection reversibly by competitively inhibiting virus binding to cells, whereas high concentrations of sCD4 block infection irreversibly by stripping gp120 from the virion surface (148, 160).

It is not known why laboratory strains acquire increased sensitivity to neutralization by sCD4 upon adaptation to growth in culture. Possibly, selective pressures which favor a more stable gp120-gp41 interaction exist in vivo. (As suggested above, this might impair binding to sCD4 and sCD4-induced shedding of gp120.) In the absence of these selective pressures, a somewhat looser gp120-gp41 association might actually favor growth in vitro (160).

Other findings suggest that shedding of gp120 may not be an important determinant of HIV inactivation by sCD4. For example, a comparison of the kinetics of sCD4 binding to virions, gp120 shedding, and HIV inactivation shows that shedding is a relatively slow process even at high sCD4 concentrations, whereas inactivation of HIV is fast. These kinetic studies suggest that the rate of reversible binding of sCD4 to HIV is the major factor underlying the inactivation of the virus, at least in vitro (57).

If some of the antiviral effect of sCD4 results from simply competing with cell surface CD4 for binding to the virion, as opposed to permanently inactivating the virion, then at least some treated virions would be able to continue encountering target cells with the possibility of eventually initiating infection. The distinction between these possible modes of action of sCD4 becomes increasingly important with increasing concentrations of target cells, since virions which have not been inactivated permanently would have more opportunities to successfully infect a cell (209). Experiments have indeed shown that the effectiveness of sCD4 against HIV decreased 20-fold as the target cell concentration was increased from 6.25×10^4 to 1.6×10^7 cells per ml (130). Concentrations of sCD4 as high as 100 μg/ml failed to block infection in vitro when the T-cell density was 5×10^7 cells per ml (58). These results show that viable HIV virions remain even after treatment with high concentrations of sCD4 and that high cell concentrations can rescue virions that have been only partially or temporarily impaired. This is clinically important for the following reasons. Whereas the concentration of T cells in the blood is about 10^6 cells per ml, the concentration in lymph nodes is about 10^8 cells per ml. Furthermore, it is now recognized that the viral burden in established HIV infections is primarily in lymphoid tissue (175). Thus, simply impairing viral attachment may not be clinically efficacious against established HIV infections.

The problems described above are compounded further by the short half-life (15 to 20 min) of sCD4 in sera (113, 197).

Variation among the members of the HIV quasispecies in vivo poses yet another difficulty for sCD4-based anti-HIV strategies. For example, two primary HIV isolates that were relatively sensitive to sCD4-induced shedding of gp120 (159) but relatively insensitive to neutralization by sCD4 were found. The insensitivity of these isolates to sCD4 appeared to result from their ability to spread by direct cell-to-cell transfer.

Despite the above provisos, sCD4 may yet prove to have important anti-HIV activity in humans. Although HIV in patients may be relatively resistant to sCD4, primary HIV isolates are, nevertheless, neutralized by high concentrations of sCD4 (13, 49). Furthermore, the maximal tolerated doses of sCD4 were not reached in the initial clinical trials. In this regard, there was early concern that rsCD4 might induce the production of antibodies reactive with CD4 on T helper cells. Although some patients did develop anti-rsCD4 antibodies, there was no apparent clinical toxicity (113). Also, the initial clinical trials involved small populations of patients and short time courses. The results of trials with higher doses of sCD4 for longer periods of time may yet show that this molecule will be useful clinically. Also, sCD4 was found to have enhanced anti-HIV activity in vitro when administered in combination with zidovudine and alpha interferon (110). Thus, it will be important to test for synergism between these agents in vivo.

Several variations of the sCD4-based anti-HIV strategy are also being evaluated. In one approach, a recombinant molecule was produced in which the two N-terminal Ig-like regions of CD4 were joined by genetic engineering to the constant (Fc) portion of an antibody molecule (36). The recombinant molecule, termed an immunoadhesin or CD4-IgG, has advantages over both anti-HIV antibodies and sCD4. A shortcoming of the natural antibodies results from the tendency of HIV to continually generate antigenic variants. However, since all viable variants of HIV must bind to CD4, CD4-IgG should interact with all HIV isolates.

CD4-IgG is more effective than sCD4 because its Fc antibody domain provides a link to immune effector functions such as antibody or complement and certain cytotoxic leukocytes. Antibody- or complement-mediated cytotoxicity normally results from the binding of antibodies to antigens expressed at the surfaces of infected cells, which in turn activates the complement system. Antibody-dependent cell-mediated cytotoxicity is normally affected by certain types of immunologically nonspecific leukocytes which bind to target cells to which antibody of the IgG class is attached. These two processes can act to prevent the spread of viral infection by destroying infected cells at early stages of infection, before many progeny virions are released. Thus, they might be mechanisms by which CD4-IgG, but not sCD4, could prevent the spread of infectious virus in vivo.

Another important feature of CD4-IgG is related to the ability of free gp120 to bind to uninfected $CD4^+$ cells. CD4-IgG, unlike natural IgG, is not able to interact with gp120 bound to an uninfected $CD4^+$ cell, presumably because gp120 has only one binding site for CD4 (36). This probably explains why CD4-IgG, unlike natural IgG, does not generate antibody-dependent cell-mediated cytotoxicity towards uninfected $CD4^+$ bystander cells that have soluble gp120 bound at their surfaces (30). Antibody-dependent cell-mediated cytotoxicity directed towards $CD4^+$ bystander cells is believed to be a mechanism of pathogenesis in AIDS.

Yet another significant advantage of CD4-IgG over sCD4 is that the Fc portion of CD4-IgG confers upon that molecule a much longer serum half-life than that of sCD4. The serum half-life of CD4-IgG is actually comparable to that of a whole immunoglobulin molecule, enabling CD4-IgG to maintain 25-fold-higher levels in the blood than sCD4 administered at equivalent doses (36).

A potentially important major advantage of CD4-IgG is that, like natural IgG, it is transferred efficiently across the primate placenta (30). This is significant because of frequent in utero

infection by HIV. Indeed, some 15 to 45% of infants born to HIV-infected mothers are themselves infected. Infection of the fetus is believed to occur late in the third trimester of pregnancy. It is hoped that it might be possible to establish sufficiently high levels of CD4-IgG in the fetus before infection from the mother can occur.

It is somewhat disquieting that primary HIV isolates are less sensitive than laboratory-adapted strains to neutralization by CD4-IgG as well as by sCD4 (160). As in the case of sCD4, sensitivity to neutralization by CD4-IgG correlated with the binding activity to intact virions. Nevertheless, there is cause for optimism in that the concentrations of CD4-IgG needed for half-maximal binding to virions and for neutralization in vitro were 5- to 50-fold lower than the concentrations needed for similar binding and neutralization by sCD4 (229). This is probably because CD4-IgG is bivalent towards gp120 on the surfaces of virions. This property, together with the higher concentrations of CD4-IgG that may be maintained in vivo and the coupling of the immunoadhesin to immune effector mechanisms, offers hope that immunoadhesin-based therapeutic strategies may be effective despite the relative resistance of primary HIV isolates.

Pretreatment of chimpanzees with CD4-IgG was found to prevent HIV infection in them (229). Under conditions in which a control animal became infected 3 weeks after challenge, the CD4-IgG-treated animals did not show any signs of infection after 47 weeks. These animals were challenged with the laboratory-adapted strain HIV-1 IIIB, which is more sensitive than primary HIV isolates to CD4-IgG. It will be interesting to know whether CD4-IgG can protect against infection by less-sensitive primary HIV isolates.

A phase I trial of CD4-IgG therapy in patients with AIDS and AIDS-related complex unfortunately did not show consistent positive effects, as would have been indicated by changes in CD4 T-cell counts or in serum p24 antigen (an HIV core protein) levels. In this clinical trial, the peak levels of serum CD4-IgG approached the concentrations needed for inhibition in vitro of some of the relatively resistant clinical HIV isolates (96). Since these levels of CD4-IgG were well tolerated by patients, there is hope that higher doses at more frequent intervals may have clinical efficacy. Furthermore, it is hoped that clinical efficacy might be significantly improved by the use of CD4-IgG in combination therapy with agents such as zidovudine, dideoxyinosine, and alpha interferon.

In another CD4-based approach to AIDS therapy, CD4 is coupled to cytotoxic molecules. This serves to target the cytotoxic component towards cells expressing HIV-encoded gp120. As in the case of antibody-dependent cell-mediated cytotoxicity mediated by CD4-IgG, cells expressing gp120 may be killed early in infection, before they produce progeny virions. In one such approach, a recombinant protein in which the gp120-binding region of CD4 was coupled to active regions of *Pseudomonas aeruginosa* exotoxin A was produced (24). In another approach, rsCD4 was coupled to the A chain of the plant toxin ricin (222). In yet another approach, bispecific antibodies were produced in which one heavy chain was coupled to rsCD4 and the other heavy/light chain pair was derived from antibody to CD3, a component of the T-cell receptor (22). These bispecific antibodies bind to cells expressing gp120, to which they induce the binding of cytotoxic T lymphocytes of any specificity.

Each of the molecules described above is strikingly effective at selectively promoting the destruction of HIV-infected cells in vitro. In the case of the hybrid between CD4 and the *P. aeruginosa* exotoxin, the 50% inhibitory concentrations for both cell killing and inhibition of virus production were minimally an order of magnitude below the K_d value for the binding of gp120 to CD4 (24). This is thought to result from the catalytic action of the hybrid toxin working intracellularly and from the fact that saturation of cell surface gp120 is not necessary for efficient killing. Each of these molecules may have therapeutic potential in vivo.

Other approaches to blocking HIV by inhibiting binding to CD4 have been tested in vitro with different measures of success. These include anti-CD4 antibodies (51, 146) as well as anti-idiotypic antibodies to anti-CD4 (90). The former blocks HIV binding, as discussed above, whereas the latter has thus far failed to mimic the neutralizing effect of sCD4. Dextran sulfate, a long-chain glucose polymer (molecular weight, approximately 8,000), which has been used as an anticoagulant or antilipemic agent, was also found to inhibit HIV in vitro, probably by blocking binding of virions to target cells or by inhibiting fusion between virions and the cell membranes (157). Since dextran sulfate inhibits HIV in vitro at concentrations that may be clinically attainable in patients, it too might have clinical potential against HIV. Synthetic peptide segments of the CD4 molecule, which might be expected to act much like sCD4, were also found to block HIV infection and inhibit syncytium formation in vitro (133). The structure of these peptides is based on amino acids 81 through 92 of CD4. It is hoped that small CD4-derived peptides with anti-HIV activity might have unique clinical potential by gaining access to organ compartments that exclude sCD4. The triphenylmethane derivative of avrin, avrintricarboxylc acid, is another small molecule of nonimmunological origin that might have clinical potential. It specifically inhibited binding of HIV and anti-CD4 MAbs to $CD4^+$ cells (196).

Receptors for Other Retroviruses

The identities of the host cell receptors for other human retroviruses, including HTLV-I, -II, and -III, are presently not known. However, they appear to be distinct from that for HIV (51). Furthermore, unlike HIV, which uses a receptor specific to the particular cell type most affected by infection, most naturally occurring retroviruses, including HTLV-I, HTLV-II, and the avian, feline, and bovine leukosis viruses, use receptors common to many cell types (51). With the exceptions that are discussed further below, the receptors for other mammalian and avian retroviruses have also not yet been characterized.

It is often possible to determine whether different retroviruses use the same receptor by making use of the observation that, when a retrovirus infects a cell, it somehow blocks infection of the cell by other related viruses that use the same receptor (230). This interference is not well understood. Presumably, the viral envelope proteins produced in infected cells bind to the receptors, thereby preventing their use by other related viruses. Interference might also result from the down-modulation of receptors from the cell surface that occurs, for example, when HIV infects T cells and monocytes. Note that this phenomenon might contribute to the development of immunodeficiency in AIDS.

On the basis of interference patterns, cross-neutralization, and host range specificities, the avian retroviruses have been classified into five subgroups (A to E) (230–232). Viruses belonging to groups B, D, and E apparently recognize closely related receptors. In contrast, the receptors for groups A and C are distinct from each other and from those for groups B, D, and E. Similarly, the murine and feline leukemia viruses were also divided into several groups on the basis of interference and competitive binding studies.

The gene encoding the receptor for an ecotropic murine leukemia virus (MuLV) was identified by DNA transfection of

nonpermissive cells (6). The base sequence of the gene shows that the receptor is a protein of 622 amino acids. Although this receptor is not similar in amino acid sequence to other known proteins, its 14 potential membrane-spanning domains are similar to those of several transporter proteins, in particular, the permeases for arginine and histidine of *Saccharomyces cerevisiae* (119). These transmembrane domains presumably form a pore through which specific molecules might pass. Indeed, *Xenopus laevis* oocytes injected with RNA transcribed from the cloned MuLV receptor gene showed increased uptake of lysine, arginine, and ornithine as well as increased binding of the gp70 MuLV envelope glycoprotein (119, 227).

MuLV provided the first demonstration of a virus using a transporter protein for its receptor. Previously identified virus receptors function at the cell surface to recognize either soluble chemical messengers or recognition proteins on the surfaces of other cells.

More recently, the cloned cDNA that confers sensitivity to infection by gibbon ape leukemia virus (GALV) was shown to be homologous to a phosphate permease of *Neurospora crassa* (107). The level of homology is sufficiently high to predict that the GALV receptor is also a transporter protein. However, the substrate that is transported is not known. GALV has been isolated from animals with lymphosarcoma or granulocytic leukemia (116). Inoculation of GALV into juvenile baboons has been shown to lead to myeloid leukemias (116a).

The GALV receptor is clearly distinct from the MuLV receptor since these receptors do not share sequence homology. However, both receptors have a similar number of membrane-spanning domains. Remarkably, a feline leukemia virus of subgroup B (FeLV-B) appears to use the same cell surface receptor that GALV does (215). Receptor cross-interference was seen when GALV or FeLV-B pseudotypes were used to superinfect cells productively infected with either GALV or FeLV-B. Furthermore, whereas murine cells are resistant to FeLV-B, murine cells expressing the human gene for the GALV receptor are susceptible to FeLV-B. Thus, a feline and a primate retrovirus apparently share the same cellular receptor. Furthermore, it is probable that an endogenous xenotropic retrovirus isolated from an Asian mouse uses this same receptor since it was shown to cross-interfere with GALV (208). The latter result is part of an extensive study of the extent to which different retroviruses might use the same receptor (see below).

Gene transfer experiments also led recently to the identification of cell surface proteins that make cells susceptible to infection by group A avian retroviruses (18). These proteins are alternative products of the same gene and contain a region closely related to the ligand-binding domain of the low-density lipoprotein receptor. There is no homology between group A Rous sarcoma virus envelope glycoproteins and the apolipoproteins that ordinarily bind to the low-density lipoprotein receptor, nor is the normal cellular function of the group A Rous sarcoma virus receptor yet known. The group A Rous sarcoma virus receptor is not related to any previously recognized retroviral receptor nor to any other known virus receptor.

Several general conclusions can be drawn from the above findings. First, taken together, these results show that closely related viruses of the same family do not necessarily use the same receptor. On the other hand, retroviruses use only a limited number of cell surface proteins for their receptors, as implied by the findings that a primate, a feline, and a xenotropic murine virus use the same receptor. A study to determine the extent to which 20 different retroviruses might use the same receptor yielded the same conclusion (208). On the basis of cross-interference (in this instance, on the ability of cells infected with different retroviruses to fuse on mixing) and pseudotype interference, the following was shown. Four monkey D-type viruses, the feline C-type virus RD114, and a baboon endogenous virus share a common receptor. Similarly, HTLV-I and HTLV-II share a common receptor with the related chimpanzee T-lymphotropic virus and macaque T-lymphotropic virus. In contrast, an amphotropic and a xenotropic murine leukemia virus, a bovine leukemia virus, and FeLV-C use unique receptors to infect human cells. Together with the finding that CD4 is the receptor for both HIV and the related simian immunodeficiency virus (193), the findings described above show that retroviruses infecting a variety of species use not fewer than eight different cell surface proteins for their receptors. Whereas it might seem that viruses of the retrovirus family use a large number of different receptors, the number of different receptors is considerably smaller than the number of different viruses that they serve.

The observations described above raise the question of what features of a cell surface protein might make it suitable for use as a viral receptor. It might be advantageous for the virus if the receptor protein were present on many cell types. However, as indicated by the HIV interaction with CD4, this is not a necessary feature of a virus receptor. The receptors for several distinctly different and unrelated viruses (e.g., poliovirus, HIV, and simian virus 40) are members of the Ig superfamily of proteins (25, 51, 120, 151). This might be fortuitous or it might reflect the number or widespread distribution of these proteins on cells. Alternatively, it might reflect a structural feature of these proteins. As noted above, the exposed loops on Ig superfamily proteins might fit into canyons on the viral surface that are smaller than the footprint of an antibody (186). This would enable the virus to undergo antigenic variation to escape neutralization, without having to alter its receptor-binding site. The canyon hypothesis is discussed in greater detail below in "Picornavirus Receptors." Another possible advantage that might be offered by a particular receptor is the ability to transmit a signal that might be important for virus entry or some postentry event (for examples, see references 22, 26, and 247). The findings that leukemia viruses of murine, primate, and feline origins use permeases for their receptors and that some members of the picornavirus family adsorb to receptors of the integrin superfamily (see below) show that other families of cell surface proteins are also suitable for use as virus receptors. The properties of these proteins that make them particularly suitable for use as virus receptors, as in the case of other virus receptors, remain to be determined.

It is not entirely clear whether the use of permease proteins as receptors by some leukemic retroviruses might have any clinical significance. The retroviral interference phenomenon seems to suggest that the receptors of infected cells are saturated with viral envelope glycoproteins. Nevertheless, the uptake of amino acids by the MuLV receptor (expressed in *X. laevis* oocytes) was not substantially affected by the viral envelope glycoprotein (227). Thus, the bound viral envelope glycoprotein does not appear to block an essential transport function. This is consistent with the ability of the virus to persistently infect a cell without compromising cell viability. Indeed, the continued presence of the virus leads to indefinite cell growth. Perhaps the binding site for the virus on the receptor is distinct from the region involved in metabolic transport.

PICORNAVIRUS RECEPTORS

Although the picornaviruses do not pose the lethal threat of HIV, they nevertheless constitute a large and important family of human pathogens. Furthermore, much is known about the interactions of some of these viruses with their receptors, and

this knowledge is now being applied to the development of antiviral therapeutic agents.

The picornavirus family includes poliovirus, human hepatitis A, coxsackievirus, and echovirus (see reference 189 for a review). Also included are the human rhinoviruses, which are responsible for up to 50% of all common colds. Although not life threatening, the common cold is the most frequent cause of virus-induced illness that receives medical attention. There are well over 100 non-cross-reactive human rhinovirus serotypes that can cause the common cold. Thus, an effective vaccine approach to controlling this illness is not likely. However, there is reason for optimism that a rational approach to drug design based on the virus-receptor interaction will result in a therapy for the common cold (149).

ICAM-1 Is the Receptor for Most Human Rhinoviruses

About 90% of the human rhinovirus serotypes (the major group) use the intercellular adhesion molecule 1 (ICAM-1) for their cell surface receptor (78, 212, 223). The remaining human rhinoviruses, with one exception, bind to another as-yet-unidentified receptor (225).

One of the research groups that identified ICAM-1 as the cell surface receptor for the major subgroup rhinoviruses first identified MAbs that inhibited rhinovirus infection (78). These MAbs were then used to isolate a 95-kDa cell surface glycoprotein from human cells that bound to rhinovirus in vitro. Sequence analysis of the isolated protein identified it as ICAM-1.

Another research group which reported that ICAM-1 is the receptor for the major subgroup rhinoviruses (212) found that viral binding is blocked by those anti-ICAM-1 MAbs that also block the interaction of ICAM-1 with its natural ligand, lymphocyte function-associated antigen 1 (LFA-1; see below). In contrast, anti-ICAM-1 MAbs that did not block the interaction with LFA-1 also did not block binding of rhinovirus. This suggested that the binding site for the major subgroup rhinoviruses on ICAM-1 is identical or close to the binding site for LFA-1. However, other results discussed below show that the interactions of rhinovirus and LFA-1 with ICAM-1 differ somewhat in their specificities.

ICAM-1 is a member of the Ig superfamily of proteins. It has five homologous extracellular Ig-like domains (D_1 to D_5, numbered sequentially from the amino end), a transmembrane domain, and a small cytoplasmic domain (211). It is structurally related to the HIV receptor CD4, which has four Ig-like domains (see above). Electron microscopy shows that ICAM-1 is a bent rod, 18.7 nm long, suggesting that the five Ig-like domains are unpaired and instead arranged head to tail (Fig. 2) (210).

ICAM-1 can be found on nasal epithelium and most other cells of the body. Its normal function is to bind to the integrin LFA-1 on the surface of lymphocytes. The LFA-1–ICAM-1 interaction mediates adhesion between cells in a wide variety of immune interactions, including the T-lymphocyte-mediated cytolysis of virally infected cells.

The surface expression of ICAM-1 is normally restricted. However, it can be induced by several mediators of inflammation, including interleukin 1, gamma interferon, and tumor necrosis factor, as well as by bacterial products released at inflammatory sites. This induction of ICAM-1 expression is probably important in localizing leukocytes to inflammatory sites. However, this induction of ICAM-1 also leads to the interesting premise that the immune response to rhinovirus infection at the site of infection might actually result in the enhanced expression of the virus receptor on nearby uninfected cells, thereby facilitating spread of the infection (212). It was also suggested that binding of rhinovirus to ICAM-1 might impede T-cell-mediated cytotoxic and helper interactions with infected cells (212). These possibilities are somewhat reminiscent of the interactions of HIV with CD4, in which there are also implications about the immune response to infection.

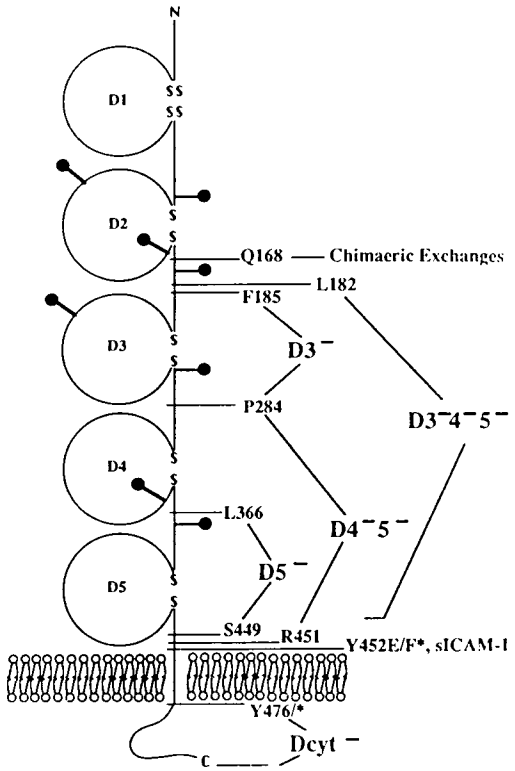

FIG. 2. ICAM-1 schematic with positions of chimeric exchanges and domain deletions. Ig-like domains are labeled D1 to D5. N-linked glycosylation sites are indicated with lollipop structures. Reprinted with permission from reference 210.

Rhinoviruses of the major group bind to cells that express primate, but not mouse, ICAM-1 (46). This finding was used to identify ICAM-1 domains important for rhinovirus binding by constructing recombinant genes encoding chimeric molecules containing human and mouse ICAM-1 sequences (Fig. 2) (210). Rhinoviruses bound well to chimeric ICAM-1 molecules containing human D_1 and D_2 domains but not to molecules containing mouse D_1 and D_2 domains. In contrast, LFA-1 bound equally well to ICAM-1 molecules irrespective of the origin of their D_1 and D_2 domains. These findings indicated that viral binding requires the sequences present in one or both of the most N-terminal Ig-like domains of ICAM-1 and that virus binding is different in its specificity from the binding of LFA-1.

The analysis of deletion mutations showed that ICAM-1 domains D_1 and D_2 are sufficient for the binding of both LFA-1 and rhinovirus, which is consistent with the findings described above (210). However, binding was diminished by deletion of D_3, D_4, or D_5. This is believed to be caused by hindrance of LFA-1 and virus from the cell surface when they attempt to bind to the truncated ICAM-1. Alternatively, diminished binding might be caused by a change in flexibility of ICAM-1, making the binding site less accessible to LFA-1 and virus.

Analysis of amino acid substitution mutations in D_1, D_2, and

D_3 further localized the binding sites for LFA-1 and rhinoviruses within D_1 (210). The studies with the ICAM-1 mutants also showed that the binding sites for LFA-1 and virus overlapped but were, nevertheless, distinct (210). Rhinovirus binding also differed from LFA-1 binding in its lack of dependence on divalent cations. As expected, residues in ICAM-1 that are important for binding of LFA-1 are more highly conserved than residues involved in binding rhinovirus.

Structure of Rhinoviruses: the Canyon Hypothesis

Rhinovirus capsids, like those of other picornaviruses, are icosahedral assemblies of 60 protomers (87). Each protomer consists of four polypeptides, VP1 to VP4. If a protomer is thought of as being triangular in shape, then VP1, VP2, and VP3 each occupy a separate corner of the triangle. VP4 is buried under each protomer, possibly in contact with the RNA genome of the virus.

In the capsid, the protomers are arranged in groups of five, called pentamers. VP1 molecules are at the vertices of the pentamers formed by each set of five protomers. In each protomer, the surface of VP1 is separated from the surfaces of VP2 and VP3 by a surface depression or canyon that is approximately 1.2 nm deep and 1.2 to 1.5 nm wide. The canyon thus separates the major part of the VP1 subunits in the "north" from the adjacent VP2 and VP3 subunits in the "south," thereby forming a moat around each pentameric vertex.

It was suggested that the canyons are the sites on the virus that bind to the cell surface receptor (186). This is based on the following. First, the large number of rhinovirus serotypes suggests that the virus undergoes extensive antigenic variation in response to antibody-driven selection. Second, the canyon is too small to permit penetration by the Fab fragments of antibody molecules which have diameters of approximately 3.5 nm. Thus, the virus can escape neutralization by varying exposed surface residues without the pressure of having to alter its crucial receptor-binding site, since the latter is inaccessible to antibody. (However, as discussed below in "Receptors for Other Picornaviruses," the conserved receptor-binding site may not have to be inaccessible to avoid neutralization by antibodies.)

The canyon hypothesis is supported by the following. First, residues lining the canyon are more conserved than residues elsewhere on the virus surface (188). The most variable surface residues are in fact at the sites of attachment of neutralizing antibodies (186, 204). The neutralizing immunogenic sites are identified by sequence analysis of viral mutants selected for their abilities to grow in the presence of neutralizing antibodies. All of the immune escape mutants are found to map on the rim of the canyon. In contrast, it was shown by site-directed mutagenesis that binding of virus to isolated receptors can be altered by changing residues in the canyon floor (47). Also, drugs that bind to a pocket beneath the canyon floor, thereby altering its topography, block the binding of rhinovirus to cell membranes and to isolated receptors (176). These drugs are discussed in greater detail below.

More recently, human rhinovirus complexed with ICAM-1 (actually a fragment containing D_1 and D_2) was examined by cryoelectron microscopy (172). Image analysis was used to generate a reconstruction of the virus-receptor complex to approximately 2.8-nm resolution. The reconstruction confirmed that ICAM-1 binds into the 1.2-nm-deep canyon on the viral surface. Furthermore, ICAM-1 appears to bind to each of the 60 symmetrically placed depressions on the virus, as predicted by the capsid structure. The ICAM-1 fragments are oriented roughly perpendicular to the virion surface. They bind to the central portion of the canyon, making more extensive contact with the southern than the northern wall and rim of the canyon. The results of the image analysis are consistent with the identification by mutational analysis of viral residues that affect binding (47). Those residues were found to be within the contact sites of ICAM-1 seen in the reconstructed images. Furthermore, the conformational changes induced by a group of antiviral agents that inhibit attachment and uncoating of certain picornaviruses by binding to a pocket beneath the canyon floor (149) are also at the exact site of ICAM-1 attachment (see below for discussion of those drugs).

The Poliovirus Receptor

Poliovirus is best known for its role in paralytic poliomyelitis. The virus infects the host after ingestion and replicates initially in lymphoid tissues of the pharynx and gut. The resulting viremia may lead to infection of the CNS in a small percentage of individuals. Viral replication within motor neurons of the brain and spinal cord results in cell death and the sequela of poliomyelitis. Note that most polioviruses are able to infect primates only.

The gene for the poliovirus receptor was isolated by use of a strategy in which nonsusceptible mouse L cells were made susceptible to poliovirus infection by transfection with a DNA library from human HeLa cells. The human PVR gene was then cloned from susceptible cells (151). The amino acid sequence of the PVR, as predicted from the DNA sequence, showed that the extracellular portion of the PVR is composed of three domains, each of which shows the conserved amino acids and domain sequence of Ig superfamily proteins. These domains are numbered sequentially from the amino-terminal end of the protein. Since the PVR does not have extensive homology with other known proteins, it is considered to be a previously unknown member of the Ig superfamily of proteins.

To identify the sequences of the PVR that are necessary for poliovirus infection, mutant PVR cDNAs were generated and transfected into mouse L cells (70). First, it was found that viral infection could occur when PVR domain 3 was deleted. Next, a chimeric receptor containing PVR domains 1 and 2, with an IgG C_H3 in place of PVR domain 3, was found to support poliovirus infection. In contrast, cells expressing chimeric PVRs lacking PVR domain 1 or 2 remained nonsusceptible to infection. This shows that each sequence within domains 1 and 2 contributes to susceptibility to infection and that the remainder of the molecule contributes in nonspecific ways. These results are reminiscent of the rhinoviruses, which required only the two most amino-terminal of the five ICAM-1 Ig-like domains for viral binding (210). Also, residues in domains 1 and 2 of CD4 each contributed to HIV infectivity (200, 228).

In another study in which mutant and chimeric forms of the PVR were expressed in mouse cells in culture (199), PVR domain 1 was sufficient for viral infectivity. However, consistent with the results described above, infectivity was enhanced by the presence of domain 2 as well. Furthermore, a hybrid molecule containing PVR domain 1 fused to ICAM-1 domains 3, 4, and 5 was found to be a functional poliovirus receptor. In addition to confirming that PVR domain 1 provides the specificity for the interaction of the PVR with poliovirus, this result is interesting with regard to the not-well-understood pathway of picornavirus entry and uncoating that leads to infection. In particular, it is not clear whether poliovirus and the major subgroup rhinoviruses follow similar pathways of entry and uncoating. However, the result with the chimeric PVR–

ICAM-1 molecule suggests that poliovirus can enter and infect cells by the ICAM-1-mediated rhinovirus pathway.

In another study involving chimeric poliovirus receptors, PVR domain 1 was attached to a truncated CD4 molecule containing CD4 domains 3 and 4 as well as the CD4 transmembrane and cytoplasmic regions (200). This chimeric molecule also conferred susceptibility to poliovirus when expressed on mouse cells. This is consistent with the study described above (200) which showed that PVR domain 1 confers receptor function for poliovirus, although the chimeric molecule was not as effective as native PVR in promoting poliovirus binding and infection. Since the transmembrane and cytoplasmic regions of the PVR, CD4, and ICAM-1 are each distinct, the results described above together show that infectious entry of poliovirus does not depend on any particular sequence not in domain 1.

Since poliovirus is nonenveloped, it is somewhat surprising that, upon binding to its receptor, it undergoes a conformational transition that is even more extensive than the binding-induced changes of enveloped HIV. The binding-induced poliovirus transition generates a 35S membrane-bound RNA-containing particle from which the internal capsid protein VP4 and the amino terminus of VP1 are extruded (71, 161). The subsequent fate of VP4 is not known. However, a mutation in VP4 resulted in a nonviable virus that could not enter cells, although it could bind to cells and generate 35S particles (161). These results imply that externalized VP4 somehow participates in virus entry after virus binding.

Like HIV, poliovirus also requires another human cell surface component in addition to its receptor for binding and entry. This was first suggested by the finding that MAb AF3 specifically inhibits poliovirus binding, although AF3 detects a 100-kDa protein that is distinct from the PVR (202). Furthermore, AF3 reacts only with cell lines and tissues that are permissive for poliovirus, whereas the PVR is also expressed in a variety of cells that poliovirus normally does not infect (69, 151, 202). AF3 was shown recently to be specific for the lymphocyte homing receptor CD44, a multifunctional glycoprotein involved in lymphocyte homing and the modulation of lymphocyte adhesion and activation (see reference 203 and references therein). It is not known whether the PVR normally interacts with CD44. It is hoped that analysis of this possible interaction might provide insights into the normal cellular function of the PVR (203). Regardless, poliovirus provides a particularly dramatic example of the dynamic nature of virus binding and entry.

The poliovirus surface, like that of the rhinoviruses, contains large depressions in the form of canyons. For this reason, and because of similarities between the PVR and ICAM-1, it is likely that the receptor-binding site on poliovirus is also in the canyon.

Receptors for Other Picornaviruses

Although conservation of viral attachment sites within virion surface depressions has been noted for mengovirus (172), a member of the cardiovirus genus of the picornavirus family, and for the hemagglutinin (183) and neuraminidase (226) surface glycoproteins of unrelated influenza virus, receptor-binding sites need not be located in small surface depressions in all instances. For example, foot-and-mouth disease virus (FMDV), a member of the apthovirus genus of the picornavirus family, lacks a canyon (1). Instead, the viral attachment site is probably located within a pronounced, highly antigenic surface loop (67). Note that the sequence Arg-Gly-Asp is a part of the receptor-binding site of FMDV VP1 (67). This sequence is a common motif in a variety of extracellular adhesion proteins (e.g., fibronectin, vitronectin, and type 1 collagen). It is part of the sequence recognized by most of the homologous members of the integrin superfamily of receptors that bind to these proteins. Thus, the as-yet-unidentified FMDV receptor may belong to the integrin supergene family of proteins. The findings described above show that in the case of the picornaviruses, as in the case of the retroviruses, members of the same virus family can use very different receptors.

Interestingly, other members of the picornavirus family also appear to use the integrin superfamily proteins for their receptors. For example, experiments screening the infection-blocking abilities of MAbs led to the identification of integrin VLA-2 as the echovirus I receptor (23). (Echovirus infections cause two-thirds of the 30,000 to 50,000 cases of viral meningitis in adolescents and children requiring hospitalization in the United States each year.) Infection was blocked by MAbs specific for either the α or β subunit of VLA-2. Unlike ICAM-1, neither of the VLA-2 subunits resembles Ig. The natural ligands for VLA-2 are the extracellular matrix proteins collagen and laminin.

Other members of the echovirus genus may use other receptors. For example, attachment of echovirus 6 is not blocked by anti-VLA-2, and this virus does not compete with echovirus I for attachment (23). Nevertheless, the unidentified echovirus 6 receptor may yet be an integrin superfamily protein.

Like poliovirus and the echoviruses, the coxsackie A viruses (CAVs) are members of the enterovirus genus of the picornavirus family. The CAVs cause a range of illnesses in humans, from a common cold-like syndrome to aseptic meningitis and paralysis. A notable feature of the CAVs is the presence of a 17-amino-acid C-terminal extension of the capsid protein, VP1, not present in the VP1 proteins of other sequenced enteroviruses (37). In each of the six CAV isolates that have been sequenced, the VP1 extension contains a similarly located Arg-Gly-Asp motif. Furthermore, the CAV VP1 extension resembles a similar sequence in FMDV. These findings imply that the Arg-Gly-Asp motif is functionally significant, most likely for binding to an integrin superfamily protein at the cell surface. The blockage of infectivity by synthetic peptides containing the Arg-Gly-Asp motif provides direct support for the role of this sequence in binding (184).

The exposed locations of the receptor-binding domains in the CAVs, as in the case of FMDV, mean that these sites are not protected from immune surveillance. However, antigenic diversity at these sites, without compromising receptor-binding specificity, may be possible if the receptor-binding sites are smaller than the footprint of an antibody (86). This may well account for the variation in the sequences flanking the Arg-Gly-Asp motif in the various CAV isolates.

It was suggested that adhesion molecules such as the integrins and ICAM-1 might be somewhat unexpected receptors for picornaviruses (86). These nonenveloped viruses are believed to enter cells by receptor-mediated endocytosis, whereas rapid endocytosis would not be an expected property of matrix and intercellular attachment proteins. Thus, attachment might be only the first step in a more elaborate sequence of events leading to virus entry into the cells. Subsequent steps might require other cell surface components in addition to the receptor per se. Such an elaborate sequence was seen in the case of related poliovirus (see above) and is characteristic of the binding and entry of the enveloped herpesviruses, as described below. Yet, in the case of the nonenveloped adenoviruses, where attachment and internalization are also distinct events, internalization, rather than binding, is dependent on integrins (235). Perhaps adhesion molecules undergo internalization when cross-linked by polyvalent virions. Alternatively, integrin

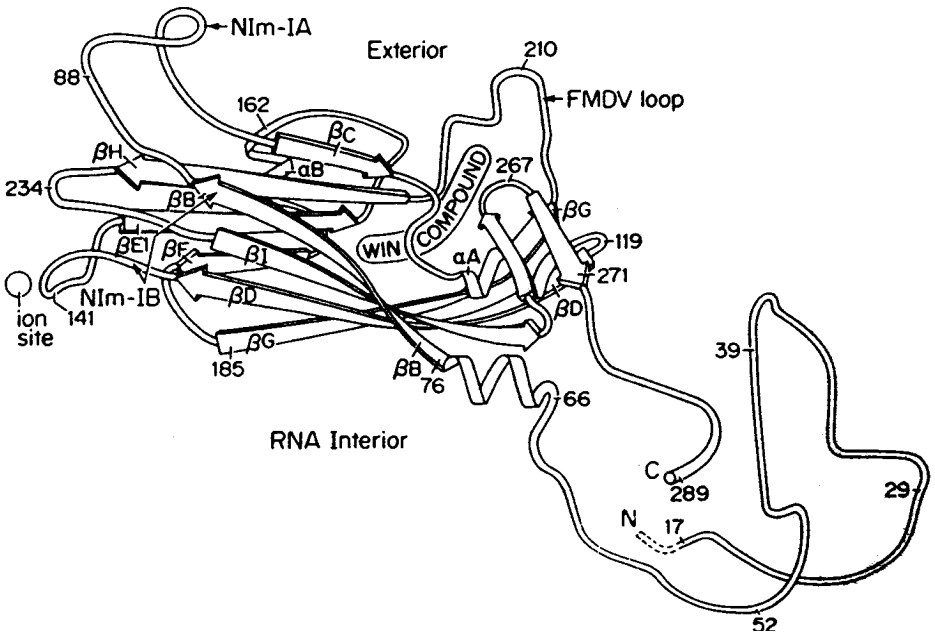

FIG. 3. Ribbon drawing of HRV14 VP1, showing the eight-stranded, antiparallel β barrel and the site of attachment for WIN 51711 in the hydrophobic internal pocket. Reprinted with permission from reference 139.

molecules are known to have an important role in cell signaling processes (reviewed in reference 102). Perhaps an integrin-mediated signal acts to promote virus entry. Also, some integrins do rapidly internalize into cells (27). Regardless, several examples in this review show that virus receptors and other cell surface components have incompletely understood but potentially interesting and important roles in virus entry subsequent to virus binding.

Receptor-Based Strategies for Controlling Rhinovirus Infections

Since common colds are caused by more than 100 distinct rhinovirus serotypes, it is highly unlikely that a vaccine that will be effective at preventing infection can be found. In contrast, since at least 78 rhinovirus serotypes use ICAM-1 for their receptor, it is possible that inhibitors of the rhinovirus–ICAM-1 interaction may be clinically useful. In support of this conjecture, an anti-ICAM-1 MAb inhibits the replication of major subgroup rhinoviruses and the development of cytopathic effects in cell culture (46). In clinical trials, intranasal inoculation with this MAb did in fact delay the emergence of symptoms by 1 or 2 days (88). Unfortunately, this treatment did not affect the frequency of infection. Hopefully, higher doses may yet be shown to have a prophylactic effect.

In another approach, purified soluble forms of ICAM-1 (sICAM-1) containing either all of the extracellular domains or domains 1 and 2 also effectively blocked replication of major subgroup rhinoviruses in cell culture (79, 143). The larger form of sICAM-1 is severalfold more effective than the smaller form at blocking rhinovirus binding. A possible explanation is that the larger molecule provides greater steric hindrance to virus binding at the cell surface.

It is not known how many of the 60 ICAM-1-binding sites on the virion need to be occupied to block infection. However, it is known that despite the 60 equivalent potential binding sites on the virus, as few as four neutralizing antibodies are sufficient to block infection by poliovirus (142). Thus, it is possible that only a few sites may need to be occupied by sICAM-1 to block infection. An additional advantage of the sICAM-1-based approach is the likelihood of the virus being unable to generate viable variants that are resistant to sICAM-1.

As mentioned above, a number of compounds are known to block either attachment or uncoating of picornaviruses (see references 149 and 187 for reviews). Among the best studied are the so-called WIN compounds. These compounds, which were derived from arildone, are each composed of a hydrophobic phenoxazole head, a seven-membered aliphatic chain, and an isoxazole tail. To appreciate how the WIN compounds interact with rhinoviruses, note that each of the picornavirus major capsid proteins, VP1, VP2, and VP3, is folded into an eight-stranded anti-parallel β-barrel motif, a common structural motif in a variety of simple RNA viruses, including viruses of insects and plants (see reference 87). Crystallographic analysis of the complex of WIN compounds with rhinovirus showed that the WIN compounds bind into a hydrophobic pocket formed by the interior of the VP1 β barrel (Fig. 3) (139). The roof of the pocket is also the floor of the canyon. Therefore, deformations of the pocket caused by drug binding result in deformations of the canyon floor and altered receptor binding as well. The WIN compounds can also block infection by inhibiting virus uncoating. As reviewed above, picornaviruses enter cells by a process that involves the extrusion of internal capsid protein VP4 and the N terminus of VP1 from the virion. The WIN compounds can inhibit these conformational changes.

Most of these antiviral drugs were identified empirically by screening the chemical libraries of pharmaceutical companies for compounds that might inhibit rhinovirus infection. This approach led to the identification of active compounds containing a variety of heterocyclic structures and alkyl chain lengths. Nevertheless, all active compounds were hydrophobic, consistent with their binding site in the virion.

Although these drugs are effective in cell culture, clinical trials have thus far been disappointing (9, 10, 177, 178, 245). Both the oral and intranasal routes of delivery were evaluated

for their abilities to protect against experimental colds with drug-sensitive virus. Since inhibitory concentrations of the drug could be reached in the blood, it is possible that the drugs never reached the site of infection. Alternatively, they may have been cleared rapidly from the site of infection. Consistent with this explanation, frequent intranasal administration (six times daily) of the drug R61837 was effective in preventing colds (8, 17). It is not yet clear whether these drugs might be useful therapeutically after the onset of symptoms. It seems unlikely that they would be, since cold symptoms are probably not dependent on the continued production of virus.

An obstacle to the approach described above for developing chemical agents effective against the rhinoviruses is the existence of a multitude of rhinovirus serotypes, each of which has a distinct pocket into which the drug must bind. Furthermore, the degrees of sensitivity of the related coxsackieviruses to WIN 54954 were found to differ as much as 100-fold among isolates of the same serotype (149). Yet another problem is the rapid emergence of virus mutants that are resistant to the WIN compounds. Mutants of HRV14, which are resistant to high concentrations of WIN 52084, can be readily selected in cell culture (91). The amino acid substitution in each of these drug-resistant mutants mapped to the drug-binding pocket. In each case, the mutation resulted in a substitution to an amino acid with a larger side chain that sterically hindered binding of the drug. Mutants of HRV14 that are resistant to low drug concentrations have also been isolated (91). Each of these mutations mapped to a site near the canyon floor rather than to the drug-binding pocket.

Although the ready emergence of drug-resistant mutants in vitro would seem to present a major impediment to the development of clinically useful drugs, the actual clinical significance of such mutants is not yet clear. Drug-resistant rhinovirus mutants were found to arise in infected individuals being treated with R61837 (52). However, the drug-resistant mutants that arose in vivo may be less virulent than the wild-type virus (5, 52). Thus, whereas drug-resistant variants remain an important concern, they do not necessarily imply that effective antiviral agents cannot be developed. A reason for being optimistic is that the rational design of antiviral agents such as the WIN compounds, based upon knowledge of the atomic structure of the rhinoviruses, is still in its infancy (187). Also, the concerns described above are probably not relevant to approaches using sICAM-1, since rhinoviruses would not be expected to become resistant to ICAM-1 derivatives while retaining the capacity to initiate infection. Thus, despite the difficulties facing researchers in this field, they continue to be optimistic that effective antiviral agents will be developed.

CORONAVIRUS RECEPTORS

Since the human coronaviruses (HCV) are another important cause of upper respiratory tract infections, recent progress in the identification of HCV receptors is noted here. One major HCV serogroup was recently shown to use human aminopeptidase N for its cell surface receptor (243). Aminopeptidase N is a cell surface glycoprotein expressed on human lung, renal, and intestinal epithelial cells and on nerve synapses (see reference 243 and references therein). It is a zinc-binding protease that catalyzes the removal of N-terminal residues from peptides. It completes the breakdown of short peptides in the gut and helps to inactivate peptide neurotransmitters in the brain.

Interestingly, transmissible gastroenteritis virus, a highly pathogenic porcine enteric coronavirus in the same serogroup as the HCVs, also uses aminopeptidase N for its receptor (54). Since aminopeptidase N is abundant at the apical surfaces of epithelial cells of the respiratory and alimentary tracts, its use as a receptor by HCVs and transmissible gastroenteritis virus provides another example of a correlation between the distribution of a receptor and the sites of viral replication and pathogenesis. Considering the receptors discussed above which are members of the Ig, integrin, and transporter superfamilies, aminopeptidase N affords yet another example of the diversity of cell surface proteins that viruses use for gaining entry into cells.

Not all HCVs use aminopeptidase N for their cell surface receptors. Recent evidence suggests that another HCV serogroup may use MHC class I antigens for its receptor (44). Mouse hepatitis coronavirus uses a carcinoembryonic antigen for its receptor (238). This protein, like MHC class I proteins, is included in the Ig superfamily. Thus, the coronaviruses offer yet another example of members of the same virus family that use several related and unrelated cell surface proteins for their receptors.

HERPESVIRUS RECEPTORS

Six types of herpesviruses have been isolated to date from humans. These include herpes simplex virus type 1 (HSV-1), HSV-2, human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), varicella-zoster virus, and human herpesvirus 6. At present, EBV is the only member of the herpesvirus family for which a high-affinity receptor has been identified definitively (see below). However, considerable effort has also gone into the analysis of the HSV and HCMV receptors. Because of the medical importance of these viruses, and the apparent complexity of their interactions with the cell surface, the virus-receptor interactions of HSV and HCMV as well as EBV are reviewed here.

HSVs

HSV-1 is responsible primarily for oral and ocular lesions causing cold sores, fever blisters, and also encephalitis. HSV-2 is responsible for genital and anal infections. Most studies of the HSV receptor have involved HSV-1. However, much of what follows may be true for HSV-2 as well.

The herpesviruses constitute another virus family that enters cells via a complex sequence of interactions at the cell surface. As in the case of the retroviruses, adsorption and penetration are distinct events involving more than one viral envelope glycoprotein and more than one cell surface component.

There have been reports that HSV can enter cells by a phagocytic process (50). However, it seems to be generally accepted that infectious entry of herpesviruses occurs through a pH-independent direct fusion of the viral envelope with the plasma membrane.

The first step in HSV infection is virion binding to heparan sulfate moieties of cell surface proteoglycans. This conclusion is based on several findings (240). First, HSV does not bind to cells from which heparan sulfate has been enzymatically removed or which do not express surface heparan sulfate because of mutation. Second, HSV can bind to immobilized heparin (which is chemically very similar to heparan sulfate). Third, soluble heparin can block HSV binding to cells.

The HSV envelope contains at least nine membrane glycoproteins. Two of these, gB and gC, show heparan sulfate-binding activity (92). However, whereas gC-deficient HSV mutants are significantly impaired in their ability to adsorb to cells, gB does not appear to be essential for this step. Nevertheless, as described below, gB and at least three other HSV

envelope glycoproteins, gD, gH, and gL, are essential for infection at a step after initial adsorption (74, 135, 185).

Initial HSV adsorption involves a plentiful cell surface component (presumably heparan sulfate proteoglycans), as shown by the difficulty in saturating binding sites. Another set of cell surface receptors, which are much more limited in number, appear necessary for HSV entry following binding (108, 109). Viral glycoprotein gD appears important in the interaction with this more limited receptor. These conclusions are based on the following. UV-inactivated wild-type virus, but not UV-inactivated gD-deficient virus, is able to block infection by untreated wild-type virus (109). However, both types of inactivated virus bind equally well to cells. These results show that gD is not required for initial adsorption but is needed instead for a step following initial adsorption. It was also found that at least 50-fold-more virus is capable of binding to the cell surface than is required to block infection (108). This shows that the virus interacts with a more limited number of receptor molecules following the initial binding event. Together, the findings described above suggest that gD interacts with the less numerous cellular component. In support of this, soluble forms of gD bind to a limited number of cell surface sites and block HSV infection but not virus binding (108). One implication of the findings described above is that, under conditions of high viral input multiplicity, most bound virions will not be able to enter cells.

Binding of HSV particles, but not of soluble gD, is dependent on heparan sulfate (108), which is consistent with the findings described above. Thus, adsorption of HSV to the numerous heparan sulfate sites may serve to facilitate its interactions via gD with a less numerous, saturable cell surface component. A more recent study found that soluble gD can interfere partially with virus binding (73), implying that gD may also be a factor in the initial binding step. However, the same study also reported that gD mediates a more stable attachment of virions to cells, as shown by the greater ease with which gD-deficient virions may be eluted from cells.

HSV envelope glycoprotein, gH, appears to function in the membrane fusion process following gD-promoted high-affinity binding as shown by the following. HSV inactivated by anti-gH antibody bound to cells and initiated, but could not complete, membrane fusion (73). Also, in contrast to the inability of gD-negative virions to block infection by standard virus (109), gH-deficient virions bound to cells and blocked adsorption of standard virus (66). These results suggest that gH acts at a step subsequent to the action of gD. Like gH, gB also appears to function primarily in the membrane fusion process rather than in adsorption, as shown by studies with temperature-sensitive mutants, anti-gH antibody-resistant mutants, and gB null mutants (31, 32, 55, 94, 136, 192). However, as noted above, the binding of soluble gB to the cell surface suggests that gB may also be involved in virus adsorption. Recent results show that glycoprotein gL is also necessary for HSV entry (185). gL appears to form a complex with gH, which is necessary for the normal folding and surface expression of gH (101).

HSV-1 is also known for its ability to invade the CNS. Recently, it was shown that both gB and gD are important determinants of HSV tropism for the CNS (105, 244). Since HSV-1 usually makes its way to the CNS via neural transmission from peripheral sites of infection, it was suggested that these virion glycoproteins facilitate infection of the CNS by promoting infection of neurons at the periphery (244).

There are reports that the fibroblast growth factor (FGF) receptor, a member of the Ig superfamily of proteins, might be a receptor for HSV. The FGF receptor was thought to mediate HSV entry by binding HSV associated with FGF (15, 114). In support of this premise, basic FGF (one of the seven currently known members of the FGF family) and a related peptide are competitive inhibitors of HSV infection. In addition, cells expressing the FGF receptor are more susceptible to HSV attachment and entry than FGF receptor-deficient cells. However, these findings were not corroborated by other investigators (156, 162, 205). In particular, null cells lacking the FGF receptor were found to be as susceptible to HSV infection as similar cells genetically engineered to express the FGF receptor. These different findings might be reconciled as follows. Cell surface heparan sulfates serve as low-affinity binding sites for basic FGF (see reference 119a for a review). Thus, basic FGF might compete with HSV for binding to heparan sulfate moieties rather than for binding to the high-affinity FGF receptor (156).

The interaction of basic FGF with heparan sulfate is particularly interesting here in that it provides an example of how growth factors, as well as viruses, may use dual receptor systems composed of low-affinity heparan sulfate proteoglycans and high-affinity classical protein-type receptors (119a). Indeed, in the absence of cell surface heparan sulfate proteoglycans, basic FGF does not bind to the high-affinity FGF receptor and is not active. The heparan sulfate moieties might concentrate basic FGF at the cell surface, thereby mediating binding of the ligand to the high-affinity receptor. The heparan sulfate might also induce changes in the conformation of basic FGF so that it might interact with its high-affinity receptor. Another possibility is that the heparan sulfate proteoglycans modulate the structure of the FGF receptor so that it might bind basic FGF. Basic FGF is probably not an isolated example of a growth factor interacting with a receptor complex rather than a single receptor molecule, since there are a number of other heparin-binding growth factors, including acidic FGF, platelet-derived growth factor, and epidermal growth factor (119a).

At present, there is no definitive evidence that identifies any non-heparan sulfate receptor for HSV. Thus, the high-affinity receptor for HSV is not yet known. There is the added complication that HSV probably makes use of multiple receptors to gain entry into cells (198). This might explain why some glycoprotein-deficient HSV mutants are only partially impaired in binding to cells and why some competitive inhibitors of HSV attachment only partially block HSV attachment to cells.

EBV

EBV is ubiquitous in humans (see reference 154 for a review). Infection can lead to acute infectious mononucleosis, a benign lymphoproliferative disease. EBV is also an oncogenic herpesvirus associated with Burkitt's lymphoma, nasopharyngeal carcinoma, and X-linked lymphoproliferative disorder. The exact role of the virus in the etiology and pathology of these malignant diseases is not yet clear, and other factors are believed to be involved in each instance. EBV also induces fatal lymphoproliferative disease, sometimes with features of frank lymphoma, in patients with congenital or acquired immunodeficiencies.

The initial site of EBV infection is the squamous epithelium of the oropharynx. B lymphocytes are also infected early during primary infection. Whereas infection of epithelial cells is productive, infection of B lymphocytes is largely latent. The epithelial cells and the B lymphocytes are the only known targets of EBV in vivo.

EBV is the only member of the herpesvirus family for which a high-affinity receptor has been identified definitively. EBV is

also unique among the herpesviruses in its selective binding to, and infection of, B lymphocytes (111, 164), as might be expected from the pathology of EBV infections. There are also reports that EBV binds to, but fails to penetrate, T cells (152).

Identification of the EBV receptor followed the realization that its host range in vitro appeared limited to primate B lymphocytes and certain epithelial cells that express CR2 (64, 112, 206), an abundant 145-kDa B-lymphocyte plasma membrane glycoprotein. CR2, also known as CD21, is the type 2 complement receptor that binds the C3d component of complement. This receptor is believed to have a role in normal B-lymphocyte activation. During the complement activation cascade, C3 is first cleaved to C3b, which may then bind covalently to antigen-antibody complexes. C3b can then be proteolytically processed to C3dg or C3d. C3d might then attach the antigen-antibody complex to the B cell via CR2, thereby helping to activate the B cell by antigen (150).

The data that establish that CR2 is the receptor for EBV include the finding that antibodies specific for CR2 block adsorption of EBV (64). Also, EBV binds to purified CR2 (167). The importance of CR2 in determining the tissue tropism for EBV is further shown by the finding that transfection of mouse L cells with the gene for human CR2 enables them to be nonproductively infected with the virus (4). In a similar manner, two human epithelial cell lines that do not express CR2 could be actively infected with EBV following transfection with a CR2 expression vector (132). These results show that the host range of EBV is determined primarily by CR2, with other host- and tissue-specific factors determining whether infection is active, latent, or abortive. Thus, the identification of CR2 as the receptor for EBV explains the tropism of EBV for B lymphocytes and the resultant pathology of the infection.

CR2 is a member of a large family of proteins that contain short consensus repeats (see reference 138 and references therein). Each short consensus repeat contains about 60 amino acids, including four invariant cysteines that are disulfide bonded to generate a structure consisting of three β strands on one face and two β strands on the other. Other members of this protein family include complement receptor type 1 (CR1/CD35), the interleukin-2 receptor, the endothelial leukocyte adhesion molecule 1 (ELAM-1), and the mouse lymph node homing receptor. At present, CR2 is the only virus receptor known to be a member of this protein family.

The CR2 extracellular domain is composed entirely of 15 or 16 short consensus repeats. There is a single transmembrane region and a cytoplasmic tail that contains 34 amino acids. The two N-terminal short consensus repeats of CR2 are necessary and sufficient to bind gp350/220 (the major envelope glycoprotein of EBV; see below) and C3dg, as shown by analysis of CR2 deletion mutants and chimeric molecules formed with CR1 (138).

Whereas the entry pathway of HSV leading to productive infection appears to be via direct membrane fusion, EBV appears to enter cells by an endocytic pathway that is somewhat unique in that the endocytic vesicles are not coated with clathrin (164). Instead, the virus is internalized into large, thin-walled vesicles. Indeed, clathrin-coated pits and vesicles in general are not observed in B cells. Another interesting, and possibly related, aspect of the EBV entry pathway is that it does not involve lysosomes, which are usually associated with receptor-mediated endocytosis via clathrin-coated vesicles. Instead, the virus appears to be released into the cytoplasm from the thin-walled vesicles. Strangely, EBV enters Raji B-lymphoblastoid cells by direct membrane fusion while also binding to these cells via CR2 (164).

EBV selectively induces endocytosis of CR2 upon binding to cells, as shown by the EBV-induced uptake of an anti-CR2 MAb, which was adsorbed to cells prior to exposure of the cells to virus (219). These results are consistent with the premise that EBV is internalized via CR2. If so, this endocytic process is not dependent on the interaction of CR2 with clathrin-coated pits. Unexpectedly, EBV failed to induce internalization of CR2 during infection of B-lymphoblastoid cells. This was the first result that suggested that EBV might enter B-lymphoblastoid cells by a process different from its entry pathway into normal B cells.

EBV differs from most other herpesviruses in that it encodes a pair of related envelope glycoproteins that predominate over the other glycoproteins in the EBV envelope. These major glycoproteins, referred to as gp350/220, are encoded by the same reading frame, with the gp220 mRNA being generated by the removal of an in-phase intron from the gp350 mRNA (see reference 216 for references). gp350/220 appears to mediate EBV binding to CR2, as shown by the ability of the purified glycoproteins to competitively inhibit EBV binding to cells (233). Also, beads coated with gp350/220 were found to adsorb to normal B lymphocytes, to specifically cap with CR2, to be endocytosed into thin-membrane vesicles, and then be released into the cytoplasm (216).

The findings described above show that gp350/220 might have a role in EBV entry after binding to the cell surface. However, another EBV envelope glycoprotein, gp85, probably plays the major role in fusion of the EBV envelope with cell membranes, as shown by the following. First, the primary amino acid sequence of gp85 is similar to that of HSV-1 envelope glycoprotein gH, which acts in fusion between the HSV-1 envelope and the plasma membrane (66, 73, 147). Second, an MAb specific for gp85 inhibits EBV fusion with cell membranes but not virus binding (155). Fusion was measured by first labeling virus with a fluorescent amphiphile probe that self-quenches at the concentrations obtained in the viral envelope. Fusion between the viral envelope and cell membranes lowers the concentration of the probe, thereby relieving the self-quenching. The kinetics of EBV fusion with lymphoblastoid cell lines and with normal lymphocytes (as monitored by the relief of self-quenching) were similar. This was somewhat surprising since EBV apparently enters lymphoblastoid cell lines by direct fusion, whereas it enters normal lymphocytes by an endocytic pathway (164, 219). However, these experiments are not able to distinguish between fusion of viral envelope with the plasma membrane and fusion with membranes of endocytic vesicles.

The following aspects of gp350/220 are of general interest and possible practical significance. gp350/220 is the primary target of neutralizing anti-EBV antibodies in humans (220). gp350/220 contains two domains homologous to sites on complement protein C3dg (166, 216), a natural ligand for CR2. A synthetic peptide corresponding to one of those domains, containing residues 16 through 29 near the N terminus of gp350/220, bound directly to purified CR2 and to CR2-expressing cells, blocked the binding of gp350/220 and C3dg to CR2 on B cells, and inhibited nearly 100% of EBV infection of B lymphocytes in vitro (165). The latter result suggests that C3dg and EBV might bind to the same site on CR2. However, there are CR2 residues required for binding EBV that are not required for binding the natural ligand, since mouse CR2 is able to bind human C3dg but not EBV (145). These results are reminiscent of data showing that mouse ICAM-1 binds human LFA-1 but not human rhinoviruses (210).

gp350/220 peptides corresponding to the other C3dg-like domain (residues 372 to 377) did not show significant CR2-binding activity (165). Whereas these studies do not eliminate

the possibility that other regions of gp350/220 might be important in EBV binding to cells, they strongly imply that the N terminus of gp350/220 mediates the binding of EBV to CR2 and the resultant infection. The identification of a major receptor-binding domain of EBV is a first step in the development of therapeutic approaches to EBV infection based on the virus-receptor interaction.

It is also interesting that gp350/220 residues 454 through 553 are colinearly homologous to amino acids 26 through 125 of HSV gC (72). The latter glycoprotein appears to play an important role in the initial interaction of HSV with its target cells.

Interferon is known to inhibit infection by EBV (221). In this regard, infection of cells by EBV is preceded by the capping of EBV-CR2 complexes at the cell surface (216). A recent study shows that the anti-EBV effect of alpha interferon may depend at least in part on its ability to inhibit capping of the EBV-CR2 complexes (53). It is suggested that this effect might be mediated by a direct interaction between alpha interferon and CR2 at the cell surface (53).

HCMV

Most HCMV infections are acquired in utero and may result in severe congenital defects (see reference 7 for a review). Although infections of other individuals can also lead to serious illness, the vast majority of HCMV infections are subclinical. This is true for infections acquired in utero, and even newborns and immunocompromised individuals can generally tolerate HCMV infections well.

Since soluble heparin was known to block adsorption of other herpesviruses to host cells, heparin was examined for its ability to block HCMV infection of skin fibroblast cells (115). Preincubation of HCMV with soluble heparin indeed prevented infection of these cells. Moreover, treatment of cells with heparinase, to remove surface heparin-like moieties, also prevented infection. Heparin affinity chromatography was used to identify glycoprotein complex II (gc-II) as the major heparin-binding component of the HCMV envelope (115). In agreement with the findings described above, other investigators (168) found that soluble heparin blocked HCMV infection of human embryonic lung cells and that the virus bound to heparin-Sepharose but not to a control Sepharose column. Also, enzymatic removal of heparan sulfate from the surfaces of human embryonic lung cells prevented infection. Furthermore, whereas HCMV was able to bind to and infect wild-type Chinese hamster ovary cells, the virus was not able to bind to and infect cell mutants deficient in heparan sulfate. Together, these results strongly suggest that HCMV, like several other herpesviruses, binds to cell surface heparin-like moieties.

Another approach to identifying the HCMV receptor was to identify cell membrane proteins that bind virus either in solution or in filter-binding assays (218). This led to the identification of proteins with a molecular mass of 30 to 34 kDa to which the virus bound predominantly in both assays. Furthermore, the amount of HCMV bound to cells after extensive washing correlated with the abundance of these proteins (169). Thus, the 30- to 34-kDa proteins might serve as a specific receptor for HCMV after initial attachment of the virus to heparan sulfate moieties in a manner similar to the two-stage binding of HSV. However, the ability of the virus to enter cells did not correlate with the abundance of these proteins (169). One possibility is that yet other cell surface components might be necessary for HCMV entry. This suggestion is supported by the following. Anti-idiotype antibodies that mimic HCMV envelope glycoprotein gp86 were generated (118). These antibodies were then used to identify a 92.5-kDa cell membrane component on human embryonic lung fibroblasts that appeared to be a specific receptor for gp86. These antibodies did not inhibit binding of HCMV to human embryonic lung cells but did inhibit fusion (117). This suggests that gp86 and its 92.5-kDa receptor might promote fusion of HCMV with the cell membrane.

It was proposed that class I proteins encoded by the MHC might be the receptor for HCMV (82). This premise was based on the following evidence. HCMV grown in cell culture binds β_2 microglobulin (β_2m; the 12-kDa light-chain component of the MHC class I heterodimer) when this protein is added to cell culture fluids or when virus is added to urine (81). Furthermore, binding of β_2m by HCMV increased viral infectivity (82). Also, HCMV and β_2m compete for binding sites on fibroblasts. Finally, significantly higher levels of β_2m-coated HCMV bound to HLA-expressing Raji cells than to HLA-negative Daudi cells (82).

The findings described above led to the proposal that β_2m-coated virions attach to surface class I heavy chains by displacing β_2m from the class I heterodimers at the cell surface (82). The ability of HCMV to bind β_2m is probably related to the finding that HCMV encodes a class I-like membrane glycoprotein (20). Nevertheless, despite the attractiveness of the hypothesis that virion-associated β_2m might mediate the specific binding of HCMV to class I proteins, it is difficult to envision how β_2m could interact simultaneously with a class I molecule on the cell surface and with the class I homolog on the virion surface (29). Furthermore, more recent results show that HCMV is able to attach to, penetrate, and initiate viral gene expression in a lymphoblastoid cell line (T2) that does not express MHC class I proteins (169). Indeed, HCMV could also bind to HLA-negative Daudi cells, although to a lesser extent than to HLA-positive Raji cells (82). Comparative studies of HCMV binding to, and infection of, lymphoid cell lines are difficult to interpret since these processes occur much less efficiently when HCMV infects lymphoid cells than when it infects fibroblasts. Nevertheless, these findings do cast doubt on the premise that MHC class I proteins are the receptor for HCMV. Doubt is sustained by the lack of correlation between levels of HLA expression on the cell surfaces of fibroblasts and the susceptibility of those cells to infection by HCMV (21). The possibility remains that HCMV binding might require a coreceptor in addition to MHC class I proteins and that the coreceptor is differentially expressed in different cell lines of various types.

Although it is not yet clear whether class I MHC proteins are a receptor for HCMV, murine cytomegalovirus has also been reported to interact with class I proteins to establish infection (241). This conclusion is based on the ability of anti-class I MAbs to block infection and on more critical studies of null cells transfected with class I alleles. Other results of that study are consistent with the possibility that extracellular β_2m enhances HCMV infection by stabilizing the conformation of surface MHC class I molecules rather than by acting as a bridge between virus and receptor.

CONCLUDING REMARKS

The virus receptors discussed above represent only a subset of the still relatively few virus receptors that have been identified to date. Also, the above examples show that the evidence establishing the identities of various virus receptors is more solid in some cases than in others. In addition, the relatively few virus-receptor interactions that have been studied extensively show that virus-receptor interactions can be complex and

dynamic, involving multiple components or sites on both the virus and the cell. Furthermore, the virus receptor not only can act as a point of attachment for the virus but also may be important in virus entry, intracellular targeting, and uncoating. In no case are the molecular details of these steps truly well understood. Thus, although we are in the midst of much basic progress in understanding virus-receptor interactions, much remains to be done.

From the clinical perspective, the identification of virus receptors has led to important insights into the pathology of infection. However, from the practical perspective, knowledge of virus-receptor interactions has not yet resulted in the development of clinically effective antiviral therapies. Despite some ingenious approaches that have been taken, and the theoretical advantages that underlie some of these strategies, therapy remains a difficult problem. One obstacle is having sufficient amounts of the drug reach the site of infection early enough to prevent the irreversible course of events that leads to disease in some cases or to prevent the spread of infection in others. Nevertheless, although efficacious receptor-based antiviral therapies may not be available immediately, investigators in this area express optimism that clinically effective agents will be developed in the near future. This optimism is based on the promise of the current basic and applied research.

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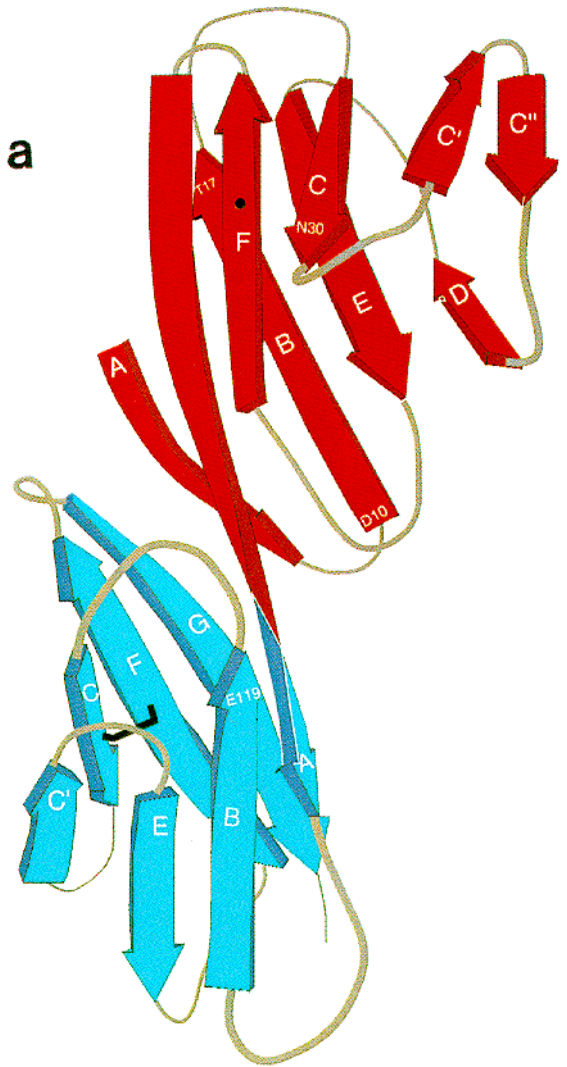
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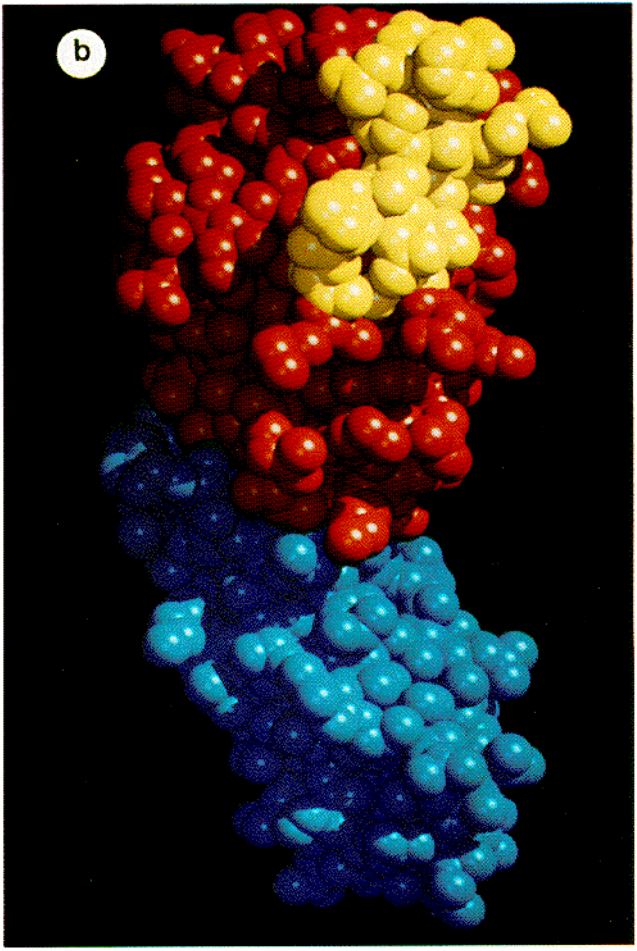
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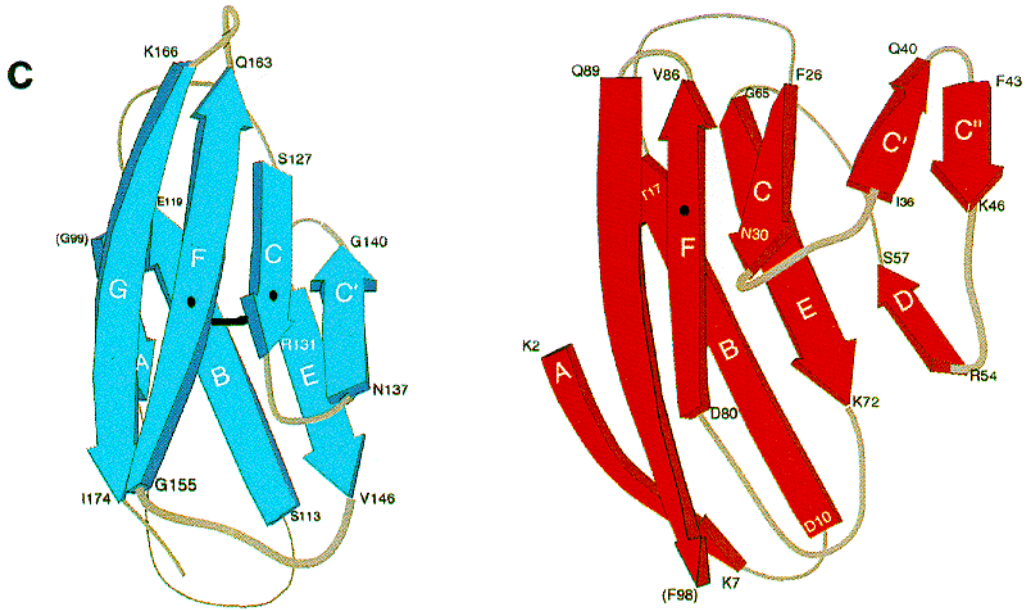
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